7. CARCINOGENICITY OF DIESEL EXHAUST

7.1. INTRODUCTION

Initial health hazard concerns regarding the potential carcinogenicity of diesel exhaust were based on the reported induction of skin papillomas by diesel particle extracts (Kotin et al., 1955), evidence for mutagenicity of extracts (Huisingh et al., 1978), evidence that components of diesel extract act as weak tumor promoters (Zamora et al., 1983), and the knowledge that diesel particles and their associated organics are respirable. During the 1980s, both human epidemiology studies and long-term animal cancer bioassays were initiated. In 1981, Waller published the first epidemiologic investigation, a retrospective mortality study of London transport workers. Since then a large number of cohort and case-control studies have been carried out with railroad workers, dockworkers, truck drivers, construction workers, and bus garage employees. During 1986 and 1987, several chronic animal cancer bioassays were published. These and numerous laboratory investigations carried out since then have been directed toward assessing the carcinogenic potential of whole exhaust, evaluating the importance of various components of exhaust in the induction of cancer, and understanding the mode of action and implications of deposition, retention, and clearance of diesel exhaust particles.

The purpose of this chapter is to evaluate the carcinogenic potential of diesel exhaust in both animals (Section 7.3) and humans (Sections 7.1 and 7.2), determine likely mode/s of action (Section 7.4), and provide an overall weight-of-evidence (Section 7.5) for carcinogenicity in humans. This assessment focuses on diesel exhaust, although diesel particles comprise a portion of all ambient particulate matter (PM). Although PM, notably PM_{10} (PM $\leq$ µm in diameter), has been identified for many years as potentially impacting human health, these effects have been evaluated in a separate document (EPA, 1996). This document is also undergoing revision.

In this section, various mortality and morbidity studies of the health effects of exposure to diesel engine emissions are reviewed. Although an attempt was made to cover all the relevant studies, a number of studies are not included for several reasons. First, the change from steam to diesel engines in locomotives began in 1935 and was about 95% complete by 1959 (Garshick et al., 1988). Diesel buses also were introduced about the same time. Therefore, exposure to diesel exhaust was less common, and the followup period for studies conducted prior to 1959 (Raffle, 1957; Kaplan, 1959) was not long enough to cover the long latency period of lung cancer. The usefulness of these studies in evaluating the carcinogenicity of diesel exhaust is greatly reduced; thus, they are not considered here.

Second, hypothesis-generating studies were excluded from this review because their findings need subsequent confirmation by definitive studies (Silverman et al., 1983; Schenker et al., 1984;

Buiatti et al., 1985; Flodin et al., 1987; Siemiatycki et al., 1988; Swanson et al., 1993; Cordier et al., 1993; Notani et al., 1993).

Third, studies in which exposure to diesel exhaust was uncertain or was defined as motor exhaust (which includes both gasoline and diesel exhaust) were excluded because they would have contributed little to the evaluation of the carcinogenicity of diesel exhaust (Waxweiler et al., 1973; Ahlberg et al., 1981; Stern et al., 1981; Vineis and Magnani, 1985; Gustafsson et al., 1986; Silverman et al., 1986; Jensen et al., 1987; Garland et al., 1988; Risch et al., 1988; Guberan et al., 1992).

Fourth, a study by Coggon et al. (1984) was not included because the occupational information abstracted from death certificates had not been validated; this would have resulted in limited information.

Three types of studies of the health effects of exposure to diesel engine emissions are reviewed in this chapter: (1) cohort studies, (2) case-control studies of lung cancer, and (3) case-control studies of bladder cancer. In the cohort studies, the cohorts of heavy construction equipment operators, railroad and locomotive workers, and bus garage employees were studied retrospectively to determine increased mortality and morbidity resulting from exposures to varying levels of diesel emissions in the workplace. A total of 9 cohort mortality studies (one of the mortality studies also included a nested lung cancer case-control study), 10 lung cancer case-control studies, and 7 bladder cancer case-control studies are considered in this section.

7.2. EPIDEMIOLOGIC STUDIES OF THE CARCINOGENICITY OF EXPOSURE TO DIESEL EXHAUST

7.2.1. Cohort Studies

7.2.1.1. *Waller (1981): Trends in Lung Cancer in London in Relation to Exposure to Diesel Fumes*

A retrospective mortality study of a cohort of London transport workers was conducted to determine if there was an excess of deaths from lung cancer that could be attributed to diesel exhaust exposure. Nearly 20,000 male employees aged 45 to 64 were followed for the 25-year period between 1950 and 1974, constituting a total of 420,700 man-years at risk. These were distributed among five job categories: drivers, garage engineers, conductors, motormen or guards, and engineers (works). Most employees lived in the greater London area. Lung cancer cases occurring in this cohort were ascertained only from death certificates of individuals who died while still employed, or if retired, following diagnosis. Expected death rates were calculated by applying greater London death rates to the population at risk within each job category. Data were calculated in 5-year periods and 5-year age ranges, finally combining the results to obtain the

total expected deaths in the required age range for the calendar period. A total of 667 cases of lung cancer was reported, compared with 849 expected, to give a mortality ratio of 79%. In each of the five job categories, the observed numbers were below those expected. Engineers in garages had the highest mortality ratio (90%), but this did not differ significantly from the other job categories. Environmental sampling was done at one garage, on 1 day in 1979, for benzo[*a*]pyrene concentrations and was compared with corresponding values recorded in 1957. Concentrations of benzo[*a*]pyrene recorded in 1957 were at least 10 times greater than those measured in 1979.

This study has several methodologic limitations. The lung cancer deaths ascertained for the study occurred while the worker was employed (the worker either died of lung cancer or retired after lung cancer was diagnosed). Although man-years at risk were based on the entire cohort, no attempt was made to trace or evaluate the individuals who had resigned from the London transport company for any other reason. Hence, information on resignees who may have had significant exposure to diesel exhaust, and lung cancer deaths among them, was not available for analysis. This fact may have led to a dilution effect, resulting in underascertainment of observed lung cancer deaths and underestimation of mortality ratios. Eligibility criteria for inclusion in the cohort, such as starting date and length of service with the company, were not specified. Because an external comparison group was used to obtain expected number of deaths, the resulting mortality ratios were less than 1; this may be a reflection of the "healthy worker effect." Investigators also did not categorize the five job categories by levels of diesel exhaust exposure, nor did they use an internal comparison group to derive risk estimates.

The age range considered for this study was limited (45 to 64 years of age) for the period between 1950 and 1964. It is not clear whether this age range was applied to calendar year 1950 or 1964 or at the midpoint of the 25-year followup period. No analyses were presented either by latency or by duration of employment (surrogate for exposure). The environmental survey based on benzo[*a*]pyrene concentrations suggests that the cohort in its earlier years was exposed to much higher concentrations of environmental contaminants than currently exist. It is not clear when the reduction in benzo[a]pyrene concentration occurred because there are no environmental readings available between 1957 and 1979. It is also important to note that the concentrations of benzo[*a*]pyrene inside the garage in 1957 were not very different from those outside the garage, thus indicating that exposure for garage workers was not much different from that of the general population. Last, no data were collected on smoking habits.

7.2.1.2. *Howe et al. (1983): Cancer Mortality (1965 to 1977) in Relation to Diesel Fumes and Coal Exposure in a Cohort of Retired Railroad Workers*

This is a retrospective cohort study of the mortality experience of 43,826 male pensioners of the Canadian National Railroad (CNR) between 1965 and 1977. Members of this cohort consisted of male CNR pensioners who had retired before 1965 and who were known to be alive at the start of that year, as well as those who retired between 1965 and 1977. The records were obtained from a computer file that is regularly updated and used by the company for payment of pensions. To receive a pension, each pensioner must provide, on a yearly basis, evidence that he is alive. Specific cause of death among members of this cohort was ascertained by linking these records to the Canadian Mortality Data Base, which contains records of all deaths registered in Canada since 1950. Of the 17,838 deaths among members of the cohort between 1965 and 1977, 16,812 (94.4%) were successfully linked to a record in the mortality file. A random sample manual check on unlinked data revealed that failure to link was due mainly to some missing information on the death records.

Occupation at time of retirement was used by the Department of Industrial Relations to classify workers into three diesel fume and coal dust exposure categories: (1) nonexposed, (2) possibly exposed, and (3) probably exposed. Person-years of observation were calculated and classified by age at observation in 5-year age groups (35 to 39, 40 to 44, . . . , 80 to 84, and $\geq$85 years). The observed deaths were classified by age at death for different cancers, for all cancers combined, and for all causes of death combined. Standard mortality ratios (SMRs) were then calculated using rates of the Canadian population for the period between 1965 and 1977.

Both total mortality (SMR = 95, $p<0.001$) and all cancer deaths (SMR = 99, $p>0.05$) were close to that expected for the entire cohort. Analysis by exposure to diesel fume levels in the three categories (nonexposed, possibly exposed, and probably exposed) revealed an increased relative risk for lung cancer among workers with increasing exposure to diesel fumes. The relative risk for nonexposed workers was presumed to be 1.0; for those possibly exposed, the relative risk was elevated to 1.2, which was statistically significant ($p=0.013$); and, for those probably exposed, it was elevated to 1.35, which was statistically highly significant ($p=0.001$). The corresponding rates for exposure to varying levels of coal dust were very similar at 1.00, 1.21 ($p=0.012$), and 1.35 ($p=0.001$), respectively. The trend tests were highly significant for both exposures ($p<0.001$). Analysis performed after the exclusion of individuals who worked in the maintenance of steam engines, and hence were exposed to high levels of asbestos, yielded the risk of lung cancer to be 1.00, 1.21, and 1.33 for those nonexposed, possibly exposed, and probably exposed to diesel exhaust, respectively, with a highly significant trend ($p<0.001$).

An analysis done on individuals who retired prior to 1950 showed the relative risk of lung cancer among nonexposed, possibly exposed, and probably exposed to be 1.00, 0.70, and 0.44, respectively, based on fewer than 15 deaths in each category. A similar analysis of individuals who retired after 1950 found the results in the same categories to be 1.00, 1.23, and 1.40,

respectively. Although retirement prior to 1950 indicated exposure to coal dust alone, retirement after 1950 shows the results of mixed exposure to coal dust and diesel fumes. As there was considerable overlap between occupations involving probable exposure to diesel fumes and probable exposure to coal dust, and as most members of the cohort were employed during the years in which the transition from coal to diesel occurred, it was difficult to distinguish whether lung cancer was associated with exposure to coal dust or diesel fumes or a mixture of both.

Although this study showed a highly significant dose-response relationship between diesel fumes and lung cancer, it has some methodological limitations. There were concurrent exposures to both diesel fumes and coal dust during the transition period; therefore, misclassification of exposure may have occurred, because only occupation at retirement was available for analysis. It is possible that the elevated response observed for lung cancer was due to the combined effects of exposure to both coal dust and diesel fumes and not just one or the other. However, it should be noted that so far coal dust has not been demonstrated to be a pulmonary carcinogen in studies of coal miners. No information was provided on duration of employment in either diesel work or the coal dust–related jobs for other than those jobs held at retirement. Therefore, it was not possible to evaluate whether this omission would have led to an under- or overestimate of the true relative risk. Furthermore, a lack of information on potential confounders such as smoking makes interpretation of the excess risk of lung cancer even more difficult. Information on cause of death was acquired from the mortality data linkage. There is a possibility that the cause of death may have been misclassified because of miscoding of the underlying cause of death.

7.2.1.3. *Rushton et al. (1983): Epidemiological Survey of Maintenance Workers in the London Transport Executive Bus Garages and Chiswick Works*

This is a retrospective mortality cohort study of male maintenance workers employed for at least 1 continuous year between January 1, 1967, and December 31, 1975, at 71 London transport bus garages (also known as rolling stock) and at Chiswick Works. For all men, the following information was obtained from computer listings: surname with initials, date of birth, date of joining company, last or present jobs, and location of work. For those individuals who left their job, date of and reason for leaving were also obtained. For those who died in service or after retirement and for men who had resigned, full name and last known address were obtained from an alphabetical card index in the personnel department. Additional tracing of individuals who had left was carried out through social security records. The area of their residence was assumed to be close to their work; therefore their place of work was coded as their residence. One hundred different job titles were coded into 20 broader groups. These 20 groups were not ranked for diesel exhaust exposure, however. The reason for leaving was coded as died in service, retired, or other. The underlying cause of death was coded using the eighth revision of the International

Classification of Diseases (ICD). Person-years were calculated from date of birth and dates of entry to and exit from the study using the man-years computer language program. These were then subdivided into 5-year age and calendar period groups. The expected number of deaths was calculated by applying the 5-year age and calendar period death rates of the comparison population with the person-years of corresponding groups. The mortality experience of the male population in England and Wales was used as the comparison population. Significance values were calculated for the difference between the observed and expected deaths, assuming a Poisson distribution.

The number of person-years of observation totaled 50,008 and was contributed by 8,490 individuals in the study with a mean followup of 5.9 years. Only 2.2% (194) of the men were not traced. Observed deaths from all causes were significantly lower than expected (observed = 495, $p<0.001$). The observed deaths from all neoplasms and cancer of the lung were approximately the same as those expected. The only significant excess observed for cancer of the liver and gall bladder at Chiswick Works was based on four deaths ($p<0.05$). A few job groups showed a significant excess of risks for various cancers. All the excess deaths observed for the various job groups, except for the general hand category, were based on very small numbers (usually smaller than five) and merited cautious interpretation. Only a notable excess in the general hand category for lung cancer was based on 48 cases (SMR = 133, $p<0.03$). However, given the fact that there was no adjustment for confounding variables such as smoking, the result should be interpreted cautiously.

This mortality study of London transport maintenance workers did not demonstrate any cancer excesses based on a large number of cases; this needs further exploration. Its limitations, including the small sample size, short duration of followup (average of only 6 years), and lack of sufficient latency period, make this study inadequate to draw any conclusions. The number of deaths by different causes and among the various job groups was too small to allow any meaningful conclusions. Details of work history were not obtained to permit any analysis by diesel exhaust exposure. Death information was ascertained from death certificates, with inherent problems of inaccuracy, misdiagnosis, and errors in coding, and it was not known whether a trained nosologist coded the death certificates. No adjustments were made for the confounding effects of smoking and socioeconomic factors.

7.2.1.4. *Wong et al. (1985): Mortality Among Members of a Heavy Construction Equipment Operators Union With Potential Exposure to Diesel Exhaust Emissions*

This is a retrospective mortality study conducted on a cohort of 34,156 male members of a heavy construction equipment operators union with potential exposure to diesel exhaust emissions. Study cohort members were identified from records maintained at Operating

Engineers' Local Union No. 3-3A in San Francisco, CA. This union has maintained both work and death records on all its members since 1964. Individuals with at least 1 year of membership in this union between January 1, 1964, and December 31, 1978, were included in the study. Work histories of the cohort were obtained from job dispatch computer tapes. The study followup period was January 1964 to December 1978. Death information was obtained from a trust fund, which provided information on retirement dates, vital status, and date of death for those who were entitled to retirement and death benefits. Approximately 50% of the cohort had been union members for less than 15 years, whereas the other 50% had been union members for 15 years or more. The average duration of membership was 15 years. As of December 31, 1978, 29,046 (85%) cohort members were alive, 3,345 (9.8%) were dead, and 1,765 (5.2%) remained untraced. Vital status of 10,505 members who had left the union as of December 31, 1978, was ascertained from the Social Security Administration. Death certificates were obtained from appropriate State health departments. Altogether, 3,243 deaths (for whom death certificates were available) in the cohort were coded using the seventh revision of the ICD. For 102 individuals, death certificates could not be obtained, only the date of death; these individuals were included in the calculation of the SMR for all causes of death but were deleted from the cause-specific SMR analyses. Expected deaths and SMRs were calculated using the U.S. national age-sex-race cause-specific mortality rates for 5-year time periods between 1964 and 1978. The entire cohort population contributed to 372,525.6 person-years in this 5-year study period.

A total of 3,345 deaths was observed, compared with 4,109 expected. The corresponding SMR for all causes was 81.4 (p=0.01), which confirmed the "healthy worker effect." A total of 817 deaths was attributed to malignant neoplasms, slightly fewer than the 878.34 expected based on U.S. white male cancer mortality rates (SMR = 93.0, p=0.05). Mostly there were SMR deficits for cause-specific cancers, including lung cancer for the entire cohort (SMR = 98.6, observed = 309). The only significant excess SMR was observed for cancer of the liver (SMR = 166.7, observed = 23, p<0.05).

Analysis by length of union membership as a surrogate of duration for potential exposure showed statistically significant increases in SMRs of cancer of the liver (SMR = 424, p<0.01) in the 10- to 14-year membership group and of the stomach (SMR = 248, p<0.05) in the 5- to 9-year membership group. No cancer excesses were observed in the 15- to 19-year and 20+-year membership groups. Although the SMR for cancer of the lung had a statistically significant deficit in the less than 5-year duration group, it showed a positive trend with increasing length of membership, which leveled off after 10 to 14 years.

Cause-specific mortality analysis by latency period showed a positive trend for SMRs of all causes of death, although all of them were statistically significant deficits, reflecting the diminishing "healthy worker effect." This analysis also demonstrated a statistically significant

SMR excess for cancer of the liver (10- to 19-year group, SMR = 257.9). The SMR for cancer of the lung showed a statistically significant deficit for a <10-year latency but showed a definite positive trend with increasing latency.

In addition to these analyses of the entire cohort, similar analyses were carried out in various subcohorts. Analyses of retirees, 6,678 individuals contributing to 32,670.1 person-years, showed statistically significant increases ($p<0.01$) in SMRs for all cancers; all causes of death; cancers of the digestive system, large intestine, respiratory system, and lung; emphysema; and cirrhosis of the liver. The other two significant excesses ($p<0.01$) were for lymphosarcoma and reticulosarcoma and nonmalignant respiratory diseases. Further analysis of the 4,075 retirees (18,677.8 person-years) who retired at age 65 or who retired earlier but had reached the age of 65 revealed statistically significant SMR increases ($p<0.05$) for all cancers, cancer of the lung, and lymphosarcoma and reticulosarcoma.

To analyze cause-specific mortality by job held (potential exposure to diesel exhaust emissions), 20 functional job titles were used, which were further grouped into three potential categories: (1) high exposure, (2) low exposure, and (3) unknown exposure. A person was classified in a job title if he ever worked on that job. Based on this classification system, if a person had ever worked in a high-exposure job title he was included in that group, even though he may have worked for a longer time in a low-exposure group or in an unknown exposure group. Information on length of work in any particular job, hence indirect information on potential length of exposure, was not available either.

For the high-exposure group a statistically significant excess was observed for cancer of the lung among bulldozer operators who had 15 to 19 years of membership and 20+ years of followup (SMR = 343.4, $p<0.05$). This excess was based on 5 out of 495 deaths observed in this group of 6,712 individuals, who contributed 80,327.6 person-years of observation.

The cause-specific mortality analysis in the low-exposure group revealed statistically significant SMR excesses in individuals who had ever worked as engineers. These excesses were for cancer of the large intestine (SMR = 807.2, observed = 3, $p<0.05$) among those with 15 to 19 years of membership and length of followup of at least 20 years, and cancer of the liver (SMR = 871.9, observed = 3, $p<0.05$) among those with 10 to 14 years of membership and length of followup of 10 to 19 years. There were 7,032 individuals who contributed to 78,402.9 person-years of observation in the low-exposure group.

For the unknown exposure group, a statistically significant SMR was observed for motor vehicle accidents only (SMR = 173.3, observed = 21, $p<0.05$). There were 3,656 individuals who contributed to 33,388.1 person-years of observation in this category.

No work histories were available for those who started their jobs before 1967 and for those who held the same job prior to and after 1967. This constituted 9,707 individuals (28% of

the cohort) contributing to 104,447.5 person-years. Statistically significant SMR excesses were observed for all cancers (SMR = 112, observed = 339, $p<0.05$) and cancer of the lung (SMR = 119.3, observed = 141, $p<0.01$). A significant SMR elevation was also observed for cancer of the stomach (SMR = 199.1, observed = 30, $p<0.01$).

This study demonstrates a statistically significant excess for cancer of the liver but also shows statistically significant deficits in cancers of the large intestine and rectum. It may be, as the authors suggested, that the liver cancer cases were actually cases resulting from metastases from the large intestine and/or rectum, since tumors of these sites will frequently metastasize to the liver. The excess in liver cancer mortality and the deficits in mortality that are due to cancer of the large intestine and rectum could also, as the authors indicate, be due to misclassification. Both possibilities have been considered by the investigators in their discussion.

Cancer of the lung showed a positive trend with length of membership as well as with latency, although none of the SMRs were statistically significant except for the workers without any work histories. The individuals without any work histories may have been the ones who were in their jobs for the longest period of time, because workers without job histories included those who had the same job before and after 1967 and thus may have worked 12 to 14 years or longer. If they had belonged to the category in which heavy exposure to diesel exhaust emissions was very common for this prolonged time, then the increase in lung cancer, as well as stomach cancer, might be linked to diesel exhaust. Further information on those without work histories should be obtained if possible because such information may be quite informative with regard to the evaluation of the carcinogenicity of diesel exhaust.

The study design is adequate, covers about a 15-year observation period, has a large enough population, and is appropriately analyzed; however, it has too many limitations to permit any conclusions. First, no exposure histories are available. One has to make do with job histories, which provide limited information on exposure level. Any person who ever worked at the job or any person working at the same job over any period of time is included in the same category; this would have a dilution effect, since extremely variable exposures were considered in the study. Second, the length of time worked in any particular job is not available. Third, work histories were not available for 9,707 individuals, who contributed 104,447.5 person-years, a large proportion of the study cohort (28%). These individuals happen to show the most evidence of a carcinogenic effect. Confounding by alcohol consumption for cancer of the liver and smoking for emphysema and cancer of the lung was not ruled out. Last, although 34,156 members were eligible for the study, the vital status of 1,765 individuals was unknown. Nevertheless, they were still considered in the denominator of all the analyses. The investigators fail to mention how the person-year calculation for these individuals was handled. Also, some of the person-years might have been overestimated, as people may have paid the dues for a particular

year and then left work. These two causes of overestimation of the denominator may have resulted in some or all the SMRs being underestimated.

As for the smoking survey, the investigators took a very small sample (133 out of 34,156, which was not even 1%). Of 133, only 107 (80%) participated. It was a systematic sample, but the authors neglected to mention how the list was prepared. Hence, the sample may not be representative of the study population and, with a small sample size, the results are not generalizable. The questionnaire asked only for current smoking history. No detailed history was obtained for the amount smoked or length of smoking history, both of which have a bearing on emphysema as well as lung carcinoma.

7.2.1.5. *Edling et al. (1987): Mortality Among Personnel Exposed to Diesel Exhaust*

This is a retrospective cohort mortality study of bus company employees, which investigated a possible increased mortality in cardiovascular diseases and cancers from diesel exhaust exposure. The cohort comprised all males employed at five different bus companies in southeastern Sweden between 1950 and 1959. Based on information from personnel registers, individuals were classified into one or more categories and could have contributed person-years at risk in more than one exposure category. The study period was from 1951 to 1983; information was collected from the National Death Registry, and copies of death certificates were obtained from the National Bureau of Statistics. Workers who died after age 79 were excluded from the study because diagnostic procedures were likely to be more uncertain at higher ages (according to investigators). The cause-, sex-, and age-specific national death rates in Sweden were applied to the 5-year age categories of person-years of observation to determine expected deaths for all causes, malignant diseases, and cardiovascular diseases. A Poisson distribution was used to calculate p-values and confidence limits for the ratio of observed to expected deaths. The total cohort of 694 men (after loss of 5 men to followup) was divided into three exposure categories: (1) clerks with lowest exposure, (2) bus drivers with moderate exposure, and (3) bus garage workers with highest exposure.

The 694 men provided 20,304 person-years of observation, with 195 deaths compared with 237 expected. A deficit in cancer deaths largely accounted for this lower-than-expected mortality in the total cohort. Among subcohorts, no difference between observed and expected deaths for total mortality, total cancers, or cardiovascular causes was observed for clerks (lowest diesel exposure), bus drivers (moderate diesel exposure), and garage workers (high diesel exposure). The risk ratios for all three categories were less than 1 except for cardiovascular diseases among bus drivers, which was 1.1.

When the analysis was restricted to members who had at least a 10-year latency period and either any exposure or an exposure exceeding 10 years, similar results were obtained, with

fewer neoplasms than expected, whereas cardiovascular diseases showed risk around or slightly above unity.

Five lung cancer deaths were observed among bus drivers who had moderate diesel exhaust exposure, whereas 7.2 were expected. The only other lung cancer death was observed among bus garage workers who had the highest diesel exhaust exposure. The small size of the cohort and poor data on diesel exhaust exposure are among the major limitations of this study. Although lifetime occupational histories were available, no industrial hygiene data were presented to validate the classification of workers into low, moderate, and high exposure to diesel exhaust based on job title. The power of the present study was estimated to be 80% to detect a relative risk of 1.2 for cardiovascular diseases and 1.4 for cancers, but for specific cancer sites, the power was much lower than this. No information was available on confounding effects of smoking and asbestos exposure at the work sites.

7.2.1.6. *Boffetta and Stellman (1988): Diesel Exhaust Exposure and Mortality Among Males in the American Cancer Society Prospective Study*

Boffetta and Stellman conducted a mortality analysis of 46,981 males whose vital status was known at the end of the first 2 years of followup. The analysis was restricted to males aged 40 to 79 years in 1982 who enrolled in the American Cancer Society's prospective mortality study of cancer. Mortality was analyzed in relation to exposure to diesel exhaust and to employment in selected occupations related to diesel exhaust exposure. In 1982, more than 77,000 American Cancer Society volunteers enrolled over 1.2 million men and women from all 50 states, the District of Columbia, and Puerto Rico in a long-term cohort study, the Cancer Prevention Study II (CPS-II). Enrollees were usually friends, neighbors, or relatives of the volunteers; enrollment was by family groups with at least one person in the household 45 years of age or older. Subjects were asked to fill out a four-page confidential questionnaire and return it in a sealed envelope. The questionnaire included history of cancer and other diseases; use of medications and vitamins; menstrual and reproductive history; occupational history; and information on diet, drinking, smoking, and other habits. The questionnaire also included three questions on occupation: (1) current occupation, (2) last occupation, if retired, and (3) job held for the longest period of time, if different from the other two. Occupations were coded to an ad hoc two-digit classification in 70 categories. Exposures at work or in daily life to any of the 12 groups of substances were also ascertained. These included diesel engine exhausts, asbestos, chemicals/acids/solvents, dyes, formaldehyde, coal or stone dusts, and gasoline exhausts. Volunteers checked whether their enrollees were alive or dead and recorded the date and place of all deaths every other year during the study. Death certificates were then obtained from State health departments and coded according to a system based on the ninth revision of the ICD by a trained nosologist.

The data were analyzed to determine the mortality for all causes and lung cancer in relation to diesel exhaust exposure, mortality for all causes and lung cancer in relation to employment in selected occupations with high diesel exhaust exposure, and mortality from other causes in relation to diesel exhaust exposure. The incidence-density ratio was used as a measure of association, and test-based confidence limits were calculated by the Miettinen method. For stratified analysis, the Mantel-Haenszel method was used for testing linear trends. Data on 476,648 subjects comprising 939,817 person-years of risk were available for analysis. Three percent of the subjects (14,667) had not given any smoking history, and 20% (98,026) of them did not give information on diesel exhaust exposure and were therefore excluded from the main diesel exhaust analysis. Among individuals who had provided diesel exhaust exposure history, 62,800 were exposed and 307,143 were not exposed. Comparison of the population with known information on diesel exhaust exposure with the excluded population with no information on diesel exhaust exposure showed that the mean ages were 54.7 and 57.7 years, the nonsmokers were 72.4% and 73.2%, and the total mortality rates per 1,000 per year were 23.0% and 28.8%, respectively.

The all-cause mortality was elevated among railroad workers (relative risk [RR] = 1.43, 95% confidence interval [CI] = 1.2, 1.72), heavy equipment operators (RR = 1.7, 95% CI = 1.19, 2.44), miners (RR = 1.34, 95% CI = 1.06, 1.68), and truck drivers (RR = 1.19, 95% CI = 1.07, 1.31). For lung cancer mortality the risks were significantly elevated for miners (RR = 2.67, 95% CI = 1.63, 4.37) and heavy equipment operators (RR = 2.60, 95% CI = 1.12, 6.06). Risks were also elevated but not significantly for railroad workers (RR = 1.59, 95% CI = 0.94, 2.69) and truck drivers (RR = 1.24, 95% CI = 0.93, 1.66). These risks were calculated according to the Mantel-Haenszel method, controlling for age and smoking. Although the relative risk was nonsignificant for truck drivers, a small dose-response effect was observed when duration of diesel exhaust exposure for them was examined. For drivers who worked for 1 to 15 years, the relative risk was 0.87, while for drivers who worked for more than 16 years, the relative risk was 1.33 (95% CI = 0.64, 2.75). Relative risks for lung cancer were not presented for other occupations. Mortality analysis for other causes and diesel exhaust exposure showed a significant excess of deaths ($p<0.05$) in the following categories: cerebrovascular disease, arteriosclerosis, pneumonia, influenza, cirrhosis of the liver, and accidents.

The two main methodologic concerns in this study are the representativeness of the study population and the quality of information on exposure. The sample, though very large, was composed of volunteers. Thus, the cohort was healthier and less frequently exposed to important risk factors such as smoking and alcohol. Self-administered questionnaires were used to obtain data on occupation and diesel exhaust exposure. None of this information was validated. Nearly 20% of the individuals had an unknown exposure status to diesel exhaust, and they experienced a

higher mortality for all causes and lung cancer than both the diesel exhaust exposed and unexposed groups. This could have introduced a substantial bias in the estimate of the association. Although only 0.8% of the subjects were lost to followup, the use of death certificates alone as a source of medical information poses problems in accuracy and coding. But the authors report that cancer deaths are routinely checked by histological confirmation from physicians or cancer registries. Given the fact that all diesel exhaust exposure occupations, such as heavy equipment operators, truck drivers, and railroad workers, showed elevated lung cancer risk, this study is suggestive of a causal association.

7.2.1.7. *Garshick et al. (1988): A Retrospective Cohort Study of Lung Cancer and Diesel Exhaust Exposure in Railroad Workers*

An earlier case-control study of lung cancer and diesel exhaust exposure in U.S. railroad workers by these investigators had demonstrated a relative odds of 1.41 (95% CI = 1.06, 1.88) for lung cancer with 20 years of work in jobs with diesel exhaust exposure. To confirm these results, a large retrospective cohort mortality study was conducted by the same investigators. Data sources for the study were the work records of the U.S. Railroad Retirement Board (RRB). The cohort was selected based on job titles in 1959, which was the year by which 95% of the locomotives in the United States were diesel powered. Diesel exhaust exposure was considered to be a dichotomous variable depending on yearly job codes between 1959 and death or retirement through 1980. Industrial hygiene evaluations and descriptions of job activities were used to classify jobs as exposed or unexposed to diesel emissions. A questionnaire survey of 534 workers at one of the railroads where workers were asked to indicate the amount of time spent in railroad locations, either near or away from sources of diesel exhaust, was used to validate this classification. Workers selected for this survey were actively employed at the time of the survey, 40 to 64 years of age, who started work between 1939 and 1949, in the job codes sampled in 1959, and were eligible for railroad benefits. To qualify for benefits, a worker must have had 10 years or more of service with the railroad and should not have worked for more than 2 years in a nonrailroad job after leaving railroad work. Workers with recognized asbestos exposure, such as repair of asbestos-insulated steam locomotive boilers, passenger cars, and steam pipes, or railroad building construction and repairs, were excluded from the job categories selected for study. However, a few jobs with some potential for asbestos exposure were included in the cohort, and the analysis was done both ways, with and without them.

The death certificates for all subjects identified in 1959 and reported by the RRB to have died through 1980 were searched. Twenty-five percent of them were obtained from the RRB and the remainder from the appropriate State departments of health. Coding of cause of death was done without knowledge of exposure history, according to the eighth revision of the ICD. If the

underlying cause of death was not lung cancer, but was mentioned on the death certificate, it was assigned as a secondary cause of death, so that the ascertainment of all cases was complete. Workers not reported by the RRB to have died by December 31, 1980, were considered to be alive. Deceased workers for whom death certificates had not been obtained or, if obtained, did not indicate cause of death, were assumed to have died of unknown causes.

Proportional hazard models were fitted that provided estimates of relative risk for death caused by lung cancer using the partial likelihood method described by Cox, and 95% confidence intervals were constructed using the asymptotic normality of the estimated regression coefficients of the proportional hazards model. Exposure was analyzed by diesel exhaust-exposed jobs in 1959 and by cumulative number of years of diesel exhaust exposure through 1980. Directly standardized rate ratios for deaths from lung cancer were calculated for diesel exhaust exposed compared with unexposed for each 5-year age group in 1959. The standardized rates were based on the overall 5-year person-year time distribution of individuals in each age group starting in 1959. The only exception to this was between 1979 and 1980, when a 2-year person-year distribution was used. The Mantel-Haenszel analogue for person-year data was used to calculate 95% confidence intervals for the standardized rate ratios.

The cohort consisted of 55,407 workers, 19,396 of whom had died by the end of 1980. Death certificates were not available for 11.7% of all deaths. Of the 17,120 deaths for whom death certificates were obtained, 48.4% were attributable to diseases of the circulatory system, whereas 21% were attributable to all neoplasms. Of all neoplasms, 8.7% (1,694 deaths) were due to lung cancer. A higher proportion of workers in the younger age groups, mainly brakemen and conductors, were exposed to diesel exhaust, while a higher proportion of workers in the older age groups were potentially exposed to asbestos. In a proportional hazards model, analyses by age in 1959 found a relative risk of 1.45 (95% CI = 1.11, 1.89) among the age group 40 to 44 years and a relative risk of 1.33 (95% CI = 1.03, 1.73) for the age group 45 to 49 years. Risk estimates in the older age groups 50 to 54, 55 to 59, and 60 to 64 years were 1.2, 1.18, and 0.99, respectively, and were not statistically significant. The two youngest age groups in 1959 had workers with the highest prevalence and longest duration of diesel exhaust exposure and lowest exposure to asbestos. When potential asbestos exposure was considered as a confounding variable in a proportional hazards model, the estimates of relative risk for asbestos exposure were all near null value and not significant. Analysis of workers exposed to diesel exhaust in 1959 (n = 42,535), excluding the workers with potential past exposure to asbestos, yielded relative risks of 1.57 (95% CI = 1.19, 2.06) and 1.34 (95% CI = 1.02, 1.76) in the 1959 age groups 40 to 44 years and 45 to 49 years. Directly standardized rate ratios were also calculated for each 1959 age group based on diesel exhaust exposure in 1959. The results obtained confirmed those obtained by using the proportional hazards model.

Relative risk estimates were then obtained using duration of diesel exhaust exposure as a surrogate for dose. In a model that used years of exposure up to and including exposure in the year of death, no exposure duration-response relationship was obtained. When analysis was done by disregarding exposure in the year of death and 4 years prior to death, the risk of dying from lung cancer increased with the number of years worked in a diesel-exhaust-exposed job. In this analysis, exposure to diesel exhaust was analyzed by exposure duration groups and in a model entering age in 1959 as a continuous variable. The workers with greater than 15 years of exposure had a relative risk of lung cancer of 1.72 (95% CI = 1.27, 2.33). The risk for 1 to 4 years of cumulative exposure was 1.20 (95% CI = 1.01, 1.44); for 5 to 9 years of cumulative exposure, it was 1.24 (95% CI = 1.06, 1.44); and for 10 to 14 years of cumulative exposure, it was 1.32 (95% CI = 1.13, 1.56). Directly standardized rate ratios were also calculated for each 1959 age group based on diesel exposure in 1959. The results obtained confirmed those obtained by using the proportional hazards model.

The results of this study, demonstrating a positive association between diesel exhaust exposure and increased lung cancer, are consistent with the results of the case-control study conducted by the same investigators in railroad workers dying of lung cancer from March 1981 through February 1982. This cohort study has addressed many of the weaknesses of the other epidemiologic studies. The large sample size (60,000) allowed sufficient power to detect small risks and also permitted the exclusion of workers with potential past exposure to asbestos. The stability of job career paths in the cohort ensured that of the workers 40 to 44 years of age in 1959 classified as diesel exhaust-exposed, 94% of the cases were still in diesel exhaust-exposed jobs 20 years later.

The main limitation of the study is the lack of quantitative data on exposure to diesel exhaust. This is one of the few studies in which industrial hygiene measurements of diesel exhaust were done. These measurements were correlated with job titles to divide the cohort in dichotomous exposure groups of exposed and nonexposed. This may have led to an underestimation of the risk of lung cancer since exposed groups included individuals with low to high exposure. The number of years exposed to diesel exhaust was used as a surrogate for dose. The dose, based on duration of employment, may have been inaccurate because individuals were working on steam or diesel locomotives during the transition period. If the categories of exposure to diesel exhaust had been set up as no, low, moderate, and high exposure, the results would have been more meaningful and so would have been the dose-response relationship. Another limitation of this study was the inability to examine the effect of years of exposure and latency. No adjustment for smoking was made in this study. However, an earlier case-control study done in the same cohort (Garshick et al., 1987) showed no significant difference in the risk estimate after adjusting for smoking. Despite these limitations, the results of this study

demonstrate that occupational exposure to diesel exhaust is associated with a modest risk (1.5) of lung cancer.

7.2.1.8. *Gustavsson et al. (1990): Lung Cancer and Exposure to Diesel Exhaust Among Bus Garage Workers*

A retrospective mortality study (from 1952 to 1986), cancer incidence study (from 1958 to 1984), and nested case-control study were conducted among a cohort of 708 male workers from five bus garages in Stockholm, Sweden, who had worked for at least 6 months between 1945 and 1970. Thirteen individuals were lost to followup, reducing the cohort to 695.

Information was available on location of workplace, job type, and beginning and ending of work periods. Workers were traced using a computerized register of the living population, death and burial books, and data from the Stockholm city archives.

For the cohort mortality analyses, death rates of the general population of greater Stockholm were used. Death rates of occupationally active individuals, a subset of the general population of greater Stockholm, were used as a second comparison group to reduce the bias from "healthy worker effect." Mortality analysis was conducted using the "occupational mortality analysis program" (OCMAP-PC). For cancer incidence analysis, the "epidemiology in Linköping" (EPILIN) program was used, with the incidence rates obtained from the cancer registry.

For the nested case-control study, both dead and incident primary lung cancers, identified in the register of cause of deaths and the cancer register, were selected as cases (20). Six controls matched on age ±2 years, selected from the noncases at the time of the diagnosis of cases, were drawn at random without replacements. Matched analyses were done to calculate odds ratios using conditional logistic regression. The EGRET and Epilog programs were used for these analyses.

Diesel exhaust and asbestos exposure assessments were performed by industrial hygienists based on the intensity of exposure to diesel exhaust and asbestos, specific for workplace, work task, and calendar time period. A diesel exhaust exposure assessment was based on (1) amount of emission (number of buses, engine size, running time, and type of fuel), (2) ventilatory equipment and air volume of the garages, and (3) job types and work practices. Based on detailed historical data and very few actual measurements, relative exposures were estimated (these were not absolute exposure levels). The scale was set to 0 for unexposed and 1 for lowest exposure, with each additional unit increase corresponding to a 50% increase in successive intensity (i.e., 1.5, 2.25, 3.38, and 5.06).

Based on personal sampling of asbestos during 1987, exposures were estimated and time-weighted annual mean exposures were classified on a scale of three degrees (0, 1, and 2). Cumulative exposures for both diesel exhaust and asbestos were calculated by multiplying the

level of exposure by the duration of every work period. An exposure index was calculated by adding for every individual contributions from all work periods for both diesel exhaust and asbestos. Four diesel exhaust index classes were created: 0 to 10, 10 to 20, 20 to 30, and >30. The four asbestos index classes were 0 to 20, 20 to 40, 40 to 60, and >60. The cumulative exposure indices were used for the nested case-control study.

Excesses were observed for all cancers and some other site-specific cancers using both comparison populations for the cohort mortality study, but none of them was statistically significant. Based on 17 cases, SMR for lung cancer were 122 and 115 using Stockholm occupationally active and general population, respectively. No dose-response was observed with increasing cumulative exposure. The cancer incidence study reportedly confirmed the mortality results (results not given).

The nested case-control study showed increasing risk of lung cancer with increasing exposure. Weighted linear regression gave RRs of 1.34 (95% CI = 1.09 to 1.64), 1.81 (95% CI = 1.20 to 2.71), and 2.43 (95% CI = 1.32 to 4.47) for the diesel exhaust indices 10 to 20, 20 to 30, and >30, respectively, using 0 to 10 as the comparison group. The study was based on 17 cases and six controls for each case matched on age ± 2 years. The results from conditional logistic regression were similar to those obtained by weighted linear regression, but none was statistically significant. Adjustment for asbestos exposure did not change the lung cancer risk for diesel exhaust.

The main strength of this study is the detailed exposure matrices constructed for both diesel exhaust and asbestos exposure, although they were based primarily on job tasks and very few actual measurements. There are a few methodological limitations to this study. The cohort is small and there were only 17 lung cancer deaths; thus the power is low. Exposure or outcome may be misclassified, although any resulting bias in the relative risk estimates is likely to be toward unity, because exposure classification was done independently of the outcome. Although the analysis by dose indices was done, no latency analysis was performed. Finally, data on smoking were missing, thus potentially confounding the lung cancer results. The authors suggest that even the heaviest smoking among individuals who were heavily exposed to diesel exhaust will be unable to explain the excess relative risk of 2.4 observed in this group. This may be an overstatement, however, as cigarette smoking is a very strong risk factor for lung cancer. Overall, this study provides some support to the excess lung cancer results found earlier among populations exposed to diesel exhaust.

7.2.1.9. *Hansen (1993): A Followup Study on the Mortality of Truck Drivers*

This is a retrospective cohort mortality study of unskilled male laborers, ages 15 to 74 years, in Denmark, identified from a nationwide census file of November 9, 1970. The exposed

group included all truck drivers employed in the road delivery or long-haul business (14,225). The unexposed group included all laborers in certain selected occupational groups considered to be unexposed to fossil fuel combustion products and to resemble truck drivers in terms of work-related physical demands and various personal background characteristics (43,024).

Through automatic record linkage between the 1970 census register (the Central Population Register 1970 to 1980) and the Death Certificate Register (1970 to 1980), the population was followed for cause-specific mortality or emigration up to November 9, 1980. Expected number of deaths among truck drivers was calculated by using the 5-year age group and 5-year time period death rates of the unexposed group and applying them to the person-years accumulated by truck drivers. ICD Revision 8 was used to code the underlying cause of death. Test-based CIs were calculated using Miettinen's method. A Poisson distribution was assumed for the smaller numbers, and CI was calculated based on exact Poisson distribution (Ciba-Geigy). Total person-years accrued by truck drivers were 138,302, whereas for the unexposed population, they were 407,780. There were 627 deaths among truck drivers and 3,811 deaths in the unexposed group. Statistically significant excesses were observed for all cancer mortality (SMR = 121, 95% CI = 104 to 140); cancer of respiratory organs (SMR = 160, 95% CI = 128 to 198), which mainly was due to cancer of bronchus and lung (SMR = 160, 95% CI = 126 to 200); and multiple myeloma (SMR = 439, 95% CI = 142 to 1,024). When lung cancer mortality was further explored by age groups, excesses were observed in most of the age groups (30 to 39, 45 to 49, 50 to 54, 55 to 59, 60 to 64, and 65 to 74), but there were small numbers of deaths in each group when stratified by age, and the excesses were statistically significant for the 55 to 59 (SMR = 229, 95% CI = 138 to 358) and 60 to 64 (SMR = 227, 95% CI = 142 to 344) age groups only.

As acknowledged by the author, the study has quite a few methodologic limitations. The exposure to diesel exhaust is assumed in truck drivers based on diesel-powered trucks, but no validation of qualitative or quantitative exposure is attempted. It is also not known whether any of these truck drivers or any other laborers had changed jobs after the census of November 9, 1970, thus creating potential misclassification bias in exposure to diesel exhaust. The lack of smoking data and a 36% rural population (usually consuming less tobacco) in the unexposed group further confound the lung cancer results. The followup period is relatively short, and a latency analysis was not attempted. At best, the findings of this study are consistent with the findings of other truck driver studies.

Table 7-1 summarizes the foregoing cohort studies.

7.2.2. Case-Control Studies of Lung Cancer

7.2.2.1. *Williams et al. (1977): Associations of Cancer Site and Type With Occupation and Industry From the Third National Cancer Survey Interview*

This paper reports findings of the analysis of the Third National Cancer Survey (TNCS). The lifetime histories, occupations, and industries were studied for associations with specific cancer sites and types after controlling for age, sex, race, education, use of cigarettes or alcohol, and geographic location. Of 13,179 cancer patients, a 10% random sample of all incident invasive cancers in eight areas, a total of 7,518 were successfully interviewed in the 3 years surveyed by the TNCS. These comprised 57% of those eligible to participate. The interview included items on use of tobacco and alcohol (by type, amount, and duration), family income, patient education, and employment history. Actual descriptions of the occupation and industry

Table 7-1. Epidemiologic studies of the health effects of exposure to diesel exhaust: cohort mortality studies

Authors	Population studied	Diesel exhaust exposure	Results	Limitations
Waller (1981)	Approximately 20,000 male London transportation workers Aged 45 to 64 years 25 years followup (1950-1974)	Five job categories used to define exposure Environmental benzo[a]pyrene concentrations measured in 1957 and 1979	SMR = 79 for lung cancer for the total cohort SMRs for all five job categories were less than 100 for lung cancer	Exposure measurement of benzo[a]pyrene showed very little difference between inside and outside the garage Incomplete information on cohort members No adjustment for confounding such as other exposures, cigarette smoking, etc. No latency analysis
Howe et al. (1983)	43,826 male pensioners of the Canadian National Railway Company Mortality between 1965 and 1977 among these pensioners was compared with mortality of general Canadian population.	Exposure groups classified by a group of experts based on occupation at the time of retirement Three exposure groups: Nonexposed Possibly exposed Probably exposed	RR = 1.2 (p=0.013) and RR = 1.3 (p=0.001) for lung cancer for possible and probable exposure, respectively A highly significant dose-response relationship demonstrated by trend test (p<0.001)	Incomplete exposure assessment due to lack of lifetime occupational history Mixed exposures to coal dust and diesel exhaust No validation of method was used to categorize exposure No data on smoking No latency analysis

Table 7-1. Epidemiologic studies of the health effects of exposure to diesel exhaust: cohort mortality studies (continued)

Authors	Population studied	Diesel exhaust exposure	Results	Limitations
Rushton et al. (1983)	8,490 male London transport maintenance workers Mortality of workers employed for 1 continuous year between January 1, 1967, and December 31, 1975, was compared with mortality of general population of England and Wales	100 different job titles were grouped in 20 broad categories The categories were not ranked for diesel exhaust exposure	SMR = 133 (p<0.03) for lung cancer in the general hand job group Several other job categories showed SS increased SMRs for several other sites based on fewer than five cases	Ill-defined diesel exhaust exposure without any ranking Average 6-year followup (i.e., not enough time for lung cancer latency) No adjustment for confounders such
Wong et al. (1985)	34,156 male heavy construction equipment operators Members of the local union for at least 1 year between January 1, 1964, and December 1, 1978	20 functional job titles grouped into three job categories for potential exposure Exposure groups (high, low, and unknown) based on job description and proximity to source of diesel exhaust emissions	SMR = 166 (p<0.05) for liver cancer for total cohort SMR = 343 (observed = 5, p<0.05) for lung cancer for high-exposure bulldozer operators with 15-19 years of membership, 20+ years of followup SMR = 119 (observed = 141, p<0.01) for workers with no work histories	No validation of exposure categories, which were based on surrogate information Incomplete employment records Employment history other than from the union not available No data on confounders such as other exposures, smoking, etc.

Table 7-1. Epidemiologic studies of the health effects of exposure to diesel exhaust: cohort mortality studies (continued)

Authors	Population studied	Diesel exhaust exposure	Results	Limitations
Edling et al. (1987)	694 male bus garage employees Followup from 1951 through 1983 Mortality of these men was compared with mortality of general population of Sweden	Three exposure groups based on job titles: High exposure, bus garage workers Intermediate exposure, bus drivers Low exposure, clerks	No SS differences were observed between observed and expected for any cancers by different exposure groups	Small sample size No validation of exposure No data on confounders such as other exposures, smoking, etc.
Boffetta and Stellman (1988)	46,981 male volunteers enrolled in the American Cancer Society=s Prospective Mortality Study of Cancer in 1982 Aged 40 to 79 years at enrollment First 2-year followup	Self-reported occupations were coded into 70 job categories Employment in high diesel exhaust exposure jobs were compared with nonexposed jobs	Total mortality (SS) elevated for railroad workers, heavy equipment operators, miners, and truck drivers Lung cancer mortality (SS) elevated for miners and heavy equipment operators Lung cancer mortality (SNS) elevated among railroad workers and truck drivers Truck drivers also showed a small dose response	Exposure information based on self-reported occupation for which no validation was done Volunteer population, probably healthy population

Table 7-1. Epidemiologic studies of the health effects of exposure to diesel exhaust: cohort mortality studies (continued)

Authors	Population studied	Diesel exhaust exposure	Results	Limitations
Garshick et al. (1988)	55,407 white male railroad workers Aged 40 to 64 years in 1959 Started work 10-20 years earlier than 1959	Industrial hygiene data correlated with job titles to dichotomize the jobs as "exposed" or "not exposed"	RR = 1.45 (40-44 year age group) RR = 1.33 (45-49 year age group) Both SS After exclusion of workers exposed to asbestos RR = 1.57 (40-44 year age group) RR = 1.34 (45-49 year age group) Both SS Dose response indicated by increasing lung cancer risk with increasing cumulative exposure	Years of exposure used as surrogate for dose Not possible to separate the effect of time since first exposure and duration of exposure
Gustavsson et al. (1990)	695 male workers from 5 bus garages in Stockholm, Sweden, who had worked for 6 months between 1945 and 1970 34 years followup (1952-1986) Nested case-control study 17 cases, six controls for each case matched on age " 2 years	Four diesel exhaust indices were created: 0 to 10, 10 to 20, 20-30, and >30 based on job tasks and duration of work	SMRs of 122 and 115 (OF and GP), respectively, SNS Case-control study results RR = 1.34 (10 to 20) RR = 1.81 (20 to 30) RR = 2.43 (>30) All SS with 0-10 as comparison group	Exposure matrix based on job tasks (not on actual measurements) Small cohort, hence low power Lack of smoking data

Table 7-1. Epidemiologic studies of the health effects of exposure to diesel exhaust: cohort mortality studies (continued)

Authors	Population studied	Diesel exhaust exposure	Results	Limitations
Hansen (1993)	Cohort of 57,249 unskilled laborers, ages 15 to 74, in Denmark (nationwide census file) November 9, 1970 Followup through November 9, 1980	Diesel exhaust exposure assumed based on diesel-powered trucks	SS SMR = 160 for bronchus and lung for total population	No actual exposure data available Lack of smoking data Job changes may have occurred from laborer to driver Short follow-up period

Abbreviations: RR = relative risk; SMR = standardized mortality ratio; SNS = statistically nonsignificant; SS = statistically significant; OF = occupationally active; GP = general population.

were recorded by interviewers and were coded separately for main lifetime employment, recent employment, and other jobs held according to the 1970 Census Coding Scheme. Occupations or industries were combined to form larger groups. Coding of occupational and industrial labels in meaningful job categories was done by one of the authors. Of the 3,539 interviewed males and 3,937 interviewed females, 95% and 84%, respectively, listed some main employment. The basic analysis consisted of an intercancer comparison and involved comparing the proportions of specific main lifetime industries and occupations among patients with cancer at one site with those of patients having cancer at other sites combined as a control group; this was done using a series of Mantel-Haenszel stratified contingency table analyses to yield odds ratios and chi-square values. Odds ratios were computed separately for males and females, controlling for age, race, education, tobacco, alcohol, and geographic location.

A total of 432 and 128 lung cancers were present in males and females, respectively. For males, an excess risk of lung cancer was observed for the following main industrial groups: mines (odds ratio [OR]= 1.21), construction (OR = 1.24), transportation (OR = 1.17), utility and sanitary services (OR = 2.79, $p<0.05$), and professional (OR = 1.41). An excess of bladder cancer was reported for the mining industry (OR = 1.61). For females, an excess of lung cancer was detected for the transportation industry (OR = 1.96); finance and retail industry (OR = 1.73); and the business, car repair, and miscellaneous service industry (OR = 2.29). None of these excesses were statistically significant. All of these odds ratios were adjusted for age, race, education, tobacco, alcohol, and geographic location. The transportation industry for males and females also showed a nonsignificant excess risk for cancers of the liver and gall bladder ducts. When the analysis was done for specific lifetime industries, the odds ratio for lung cancer in males was 1.40 for railroad workers and 1.34 for truck drivers. Both of these excesses were statistically nonsignificant.

The strengths of the TNCS interview data set are its large size, histological confirmation of nearly 95% of diagnoses, availability of information on occupation, and details of confounding variables obtained by personal interview and ability to control for them. Among its weaknesses are a 47% nonresponse rate and the fact that the population surveyed came from predominantly urban areas and did not represent many industries. Also, most of the associations observed did not achieve statistical significance because they were based on small numbers of patients who had both specific cancers and specific types of employment. The control group was the combined "other cancers," which may have diluted the association because diesel exhaust is also suspected of being associated with bladder cancer, and this category was included in the control group when the comparison was made with lung cancer. The study presented several tables, but the total population in each table was different and never added up to the initial number interviewed. The

authors failed to explain these omissions. Furthermore, when multiple comparisons are made, some significant associations arise by chance. This analysis suggests an association with lung cancer for three industries with potential diesel exhaust exposure: trucking, railroading, and mining.

7.2.2.2. *Hall and Wynder (1984): A Case-Control Study of Diesel Exhaust Exposure and Lung Cancer*

Hall and Wynder (1984) conducted a case-control study of 502 male lung cancer cases and 502 controls without tobacco-related diseases that examined an association between occupational diesel exhaust exposure and lung cancer. Histologically confirmed primary lung cancer patients who were 20 to 80 years old were ascertained from 18 participating hospitals in six U.S. cities, 12 months prior to the interview. Eligible controls, patients at the same hospitals without tobacco-related diseases, were matched to cases by age (±5 years), race, hospital, and hospital room status. The number of male lung cancer cases interviewed totaled 502, which was 64% of those who met the study criteria for eligibility. Of the remaining 36%, 8% refused, 21% were too ill or had died, and 7% were unreliable. Seventy-five percent of eligible controls completed interviews. Of these interviewed controls, 49.9% were from the all-cancers category, whereas 50.1% were from the all-noncancers category. All interviews were obtained in hospitals to gather detailed information on smoking history, coffee consumption, artificial sweetener use, residential history, and abbreviated medical history as well as standard demographic variables. Occupational information was elicited by a question on the usual lifetime occupation and was coded by the abbreviated list of the U.S. Bureau of Census Codes. The odds ratios were calculated to evaluate the association between diesel exhaust exposure and risk of lung cancer incidence. Summary odds ratios were computed by the Mantel-Haenszel method after adjusting for potential confounding by age, smoking, and socioeconomic class. Two-sided, 95% confidence intervals were computed by Woolf's method. Occupational exposure to diesel exhaust was defined by two criteria. First, occupational titles were coded "probably high exposure" as defined by the industrial hygiene standards established for the various jobs. The job titles included under this category were warehousemen, bus and truck drivers, railroad workers, and heavy equipment operators and repairmen. The second method used the National Institute for Occupational Safety and Health (NIOSH) criteria to analyze occupations by diesel exposure. In this method, the estimated proportion of exposed workers was computed for each occupational category by using the NIOSH estimates of the exposed population as the numerator and the estimates of individuals employed in each occupational category from the 1970 census as the denominator. Occupations estimated to have at least 20% of their employees exposed to diesel

exhaust were defined as "high exposure," those with 10% to 19% of their employees exposed were defined as "moderate exposure," and those with less than 10% of their employees exposed were defined as "low exposure."

Cases and controls were compared with respect to exposure. The relative risk was 2.0 (95% CI = 1.2, 3.2) for those workers who were exposed to diesel exhaust versus those who were not. The risk, however, decreased to a nonsignificant 1.4 when the data were adjusted for smoking. Analysis by NIOSH criteria found a nonsignificant relative risk of 1.7 in the high-exposure group. There were no significantly increased cancer risks by occupation either by the first method or by the NIOSH method. To assess any possible synergism between diesel exhaust exposure and smoking, the lung cancer risks were calculated for different smoking categories. The relative risks were 1.46 among nonsmokers and ex-smokers, 0.82 among current smokers of <20 cigarettes/day, and 1.3 among current smokers of 20+ cigarettes/day, indicating a lack of synergistic effects.

The major strength of this study is the availability of a detailed smoking history for all the study subjects. However, this is offset by lack of diesel exhaust exposure measurements, use of a poor surrogate for exposure, and lack of consideration of latency period. Information was collected on only one major lifetime occupation, and it is likely that those workers who had more than one major job may not have reported the occupation with the heaviest diesel exhaust exposures. Furthermore, occupational histories were obtained from self-reports and were not validated with work records. This could have resulted in recall bias and misclassification of exposure status.

7.2.2.3. *Damber and Larsson (1987): Occupation and Male Lung Cancer, a Case-Control Study in Northern Sweden*

A case-control study of lung cancer was conducted in northern Sweden to determine the occupational risk factors that could explain the large geographic variations of lung cancer incidence in that country. The study region comprised the three northernmost counties of Sweden, with a total male population of about 390,000. The rural municipalities with 15% to 20% of the total population have forestry and agriculture as dominating industries, and the urban areas have a variety of industrial activities (mines, smelters, steel factories, paper mills, and mechanical workshops). All male cases of lung cancer reported to the Swedish Cancer Registry during the 6-year period between 1972 and 1977 who had died before the start of the study were selected. Of 604 eligible cases, 5 did not have microscopic confirmation and in another 5 the diagnosis was doubtful, but these cases were included nevertheless. Cases were classified as small carcinomas, squamous cell carcinomas, adenocarcinomas, and other types. For each case a dead

control was drawn from the National Death Registry matched by sex, year of death, age, and municipality. Deaths in controls classified as lung cancer and suicides were excluded. A living control matched to the case by sex, year of birth, and municipality was also drawn from the National Population Registry. Postal questionnaires were sent to close relatives of cases and dead controls, and to living controls themselves to collect data on occupation, employment, and smoking habits. Replies were received from 589 cases (98%), 582 surrogates of dead controls (96%), and 453 living controls (97%).

Occupational data were collected on occupations or employment held for at least 1 year and included type of industry, company name, task, and duration of employment. Supplementary telephone interviews were performed if occupational data were lacking for any period between age 20 and time of diagnosis. Data analysis involved calculation of the odds ratios by the exact method based on the hypergeometric distribution and the use of a linear logistic regression model to adjust for the potential confounding effects of smoking. Separate analyses were performed with dead and living controls, and on the whole there was good agreement between the two control groups. A person who had been active for at least 1 year in a specific occupation was in the analysis assigned to that occupation.

Using dead controls, the odds ratios adjusted for smoking were 1.0 (95% CI = 0.7, 1.5) and 2.7 (95% CI = 1.0, 8.1) for professional drivers ($\geq$1 year of employment) and underground miners ($\geq$1 year of employment), respectively. For 20 or more years of employment in those occupations, the odds ratios adjusted for smoking were 1.2 (95% CI = 0.6, 2.2) and 9.8 (95% CI = 1.5, 414). These were the only two occupations listed with potential diesel exhaust exposure. An excess significant risk was detected for copper smelter workers, plumbers, and electricians, as well as concrete and asphalt workers. Occupational asbestos exposure was also associated with an elevated odds ratio of 2.6 (95% CI = 1.6, 3.6) for $\geq$1 year of employment and 3.6 (95% CI = 1.9, 7.2) for $\geq$20 years of employment. All the odds ratios were calculated by adjusting for age, smoking, and municipality. After comparison with the live controls, the odds ratios were found to be lower than those observed with dead controls. None of the odds ratios were statistically significant in this comparison.

This study did not detect any excess risk of lung cancer for professional drivers, who, among all the occupations listed, had the most potential for exposure to motor vehicle exhaust. However, it is not known whether these drivers were exposed exclusively to gasoline exhaust, diesel exhaust, or varying degrees of both. An excess risk was detected for underground miners, but it is not known if this was due to diesel emissions from engines or from radon daughters in poorly ventilated mines. Although a high response rate (98%) was obtained by the postal

questionnaires, the use of surrogate respondents is known to lead to misclassification errors that can bias the odds ratio to 1.

7.2.2.4. *Lerchen et al. (1987): Lung Cancer and Occupation in New Mexico*

This is a population-based case-control study conducted in New Mexico that examined the association between occupation and occurrence of lung cancer in Hispanic and non-Hispanic whites. Cases involved residents of New Mexico, 25 through 84 years of age and diagnosed between January 1, 1980, and December 31, 1982, with primary lung cancer, excluding bronchioalveolar carcinoma. Cases were ascertained through the New Mexico Tumor Registry, which is a member of the Surveillance Epidemiology and End Results (SEER) Program of the National Cancer Institute. Controls were chosen by randomly selecting residential telephone numbers and, for those over 65 years of age, from the Health Care Financing Administration's roster of Medicare participants. They were frequency-matched to cases for sex, ethnicity, and 10-year age category with a ratio of 1.5 controls per case. The 506 cases (333 males and 173 females) and 771 controls (499 males and 272 females) were interviewed, with a nonresponse rate of 11% for cases. Next of kin provided interviews for 50% and 43% of male and female cases, respectively. Among controls, only 2% of the interviews were provided by next of kin for each sex. Data were collected by personal interviews conducted by bilingual interviewers in the participants' homes. A lifetime occupational history and a self-reported history of exposure to specific agents were obtained for each job held for at least 6 months since age 12. Questions were asked about the title of the position, duties performed, location and nature of industry, and time at each job title. A detailed smoking history was also obtained. The variables on occupational exposures were coded according to the Standard Industrial Classification scheme by a single person and reviewed by another. To test the hypothesis about the high-risk jobs for lung cancer, an a priori listing of suspected occupations and industries was created by a two-step process involving a literature review for implicated industries and occupations by the principal investigator. The appropriate Standard Industrial Classification and Standard Occupational Codes associated with job titles were also determined by the principal investigator. For four agents—asbestos, wood dust, diesel exhaust, and formaldehyde—the industries and occupations determined to have exposure were identified, and linking of specific industries and occupations was based on literature review and consultation with local industrial hygienists.

The relative odds were calculated for suspect occupations and industries, classifying individuals as ever employed for at least 1 year in an industry or occupation and defining the reference group as those subjects never employed in that particular industry or occupation. Multiple logistic regression models were used to control simultaneously for age, ethnicity, and

smoking status. For occupations with potential diesel exhaust exposure, the analysis showed no excess risks for diesel engine mechanics and auto mechanics. Similarly, when analyzed by exposure to specific agents, the odds ratio adjusted for age, smoking, and ethnicity was not elevated for diesel exhaust fumes (OR = 0.6, 95% CI = 0.2, 1.6). Elevated odds ratios were found for uranium miners (OR = 2.8, 95% CI = 1.0, 7.7), underground miners (OR = 2.4, 95% CI = 1.2, 4.4), construction painters (OR = 2.4, 95% CI = 0.6, 9.6), and welders (OR = 4.3, 95% CI = 1.6, 11.0). No excess risks were detected for the following industries: shipbuilding, petroleum refining, construction, printing, blast furnace, and steel mills. No excess risks were detected for the following occupations: construction workers, painters, plumbers, paving equipment operators, roofers, engineers and firemen, woodworkers, and shipyard workers. Females were excluded from detailed analysis because none of the Hispanic female controls had been employed in high-risk jobs; among the non-Hispanic white controls, employment in a high-risk job was recorded for at least five controls for only two industries, construction and painting, for which the odds ratios were not significantly elevated. Therefore, the analyses were presented for males only.

Among the many strengths of this study are its population-based design, high participation rate, detailed smoking history, and the separate analysis done for the two ethnic groups, southwestern Hispanic and non-Hispanic white males. The major limitations pertain to the occupational exposure date. Job titles obtained from occupational histories were used as proxy for exposure status, but these were not validated. Further, for nearly half the cases, next of kin provided occupational histories. The authors acknowledge the above sources of bias but state without substantiation that these biases would not strongly affect their results. They also did not use a job exposure matrix to link occupations to exposures and did not provide details on the method they used to classify individuals as diesel exhaust exposed based on reported occupations. The observed absence of an association for exposure to asbestos, a well-established lung carcinogen, may be explained by the misclassification errors in exposure status or by sample size constraints (not enough power). Likewise, the association for diesel exhaust reported by only 7 cases and 17 controls also may have gone undetected because of low power. In conclusion, there is insufficient evidence from this study to confirm or refute an association between lung cancer and diesel exhaust exposure.

7.2.2.5. *Garshick et al. (1987): A Case-Control Study of Lung Cancer and Diesel Exhaust Exposure in Railroad Workers*

An earlier pilot study of the mortality of railroad workers by the same investigators (Schenker et al., 1984) found a moderately high risk of lung cancer among the workers who were

exposed to diesel exhaust compared with those who were not. This study was designed to evaluate the feasibility of conducting a large retrospective cohort study. On the basis of these findings the investigators conducted a case-control study of lung cancer in the same population. The population base for this case-control study was approximately 650,000 active and retired male U.S. railroad workers with 10 years or more of railroad service who were born in 1900 or later. The U.S. Railroad Retirement Board (RRB), which operates the retirement system, is separate from the Social Security System, and to qualify for the retirement or survivor benefits the workers had to acquire 10 years or more of service. Information on deaths that occurred between March 1, 1981, and February 28, 1982, was obtained from the RRB. For 75% of the deceased population, death certificates were obtained from the RRB, and, for the remaining 25%, they were obtained from the appropriate state departments of health. Cause of death was coded according to the eighth revision of the ICD. The cases were selected from deaths with primary lung cancer, which was the underlying cause of death in most cases. Each case was matched to two deceased controls whose dates of birth were within 2.5 years of the date of birth of the case and whose dates of death were within 31 days of the date of death noted in the case. Controls were then selected randomly from workers who did not have cancer noted anywhere on their death certificates and who did not die of suicide or of accidental or unknown causes.

Each subject's work history was determined from a yearly job report filed by his employer with the RRB from 1959 until death or retirement. The year 1959 was chosen as the effective start of diesel exhaust exposure for this study, since by this time 95% of the locomotives in the United States were diesel powered. Investigators acknowledge that because the transition to diesel-powered engines took place in the early 1950s, some workers had additional exposure prior to 1959; however, if a worker had died or retired prior to 1959, he was considered unexposed. Exposure to diesel exhaust was considered to be dichotomous for this study, which was assigned based on an industrial hygiene evaluation of jobs and work areas. Selected jobs with and without regular diesel exhaust exposure were identified by a review of job title and duties. Personal exposure was assessed in 39 job categories representative of workers with and without diesel exhaust exposure. Those jobs for which no personal sampling was done were considered exposed or unexposed on the basis of similarities in job activities and work locations and by degree of contact with diesel equipment. Asbestos exposure was categorized on the basis of jobs held in 1959, or on the last job held if the subject retired before 1959. Asbestos exposure in railroads occurred primarily during the steam engine era and was related mostly to the repair of locomotive steam boilers that were insulated with asbestos. Smoking history information was obtained from the next of kin.

Death certificates were obtained for approximately 87% of the 15,059 deaths reported by the RRB, from which 1,374 cases of lung cancer were identified. Fifty-five cases of lung cancer were excluded from the study for either incomplete data (20) or refusal by two States to use information on death certificates to contact the next of kin. Successful matching to at least one control with work histories was achieved for 335 (96%) cases ≤64 years of age at death and 921 (95%) cases ≥65 years of age at death. In both age groups, 90% of the cases were matched with two controls. There were 2,385 controls in the study, 98% were matched within ±31 days of the date of death, whereas the remaining 2% were matched within 100 days. Deaths from diseases of the circulatory system predominated among controls. Among the younger workers, approximately 60% had exposure to diesel exhaust, whereas among older workers, only 47% were exposed to diesel exhaust.

Analysis by a regression model, in which years of diesel exhaust exposure were the sum total of the number of years in diesel-exposed jobs, used as a continuous exposure variable, yielded an odds ratio of lung cancer of 1.39 (95% CI = 1.05, 1.83) for over 20 years of diesel exhaust exposure in the ≤64 years of age group. After adjustment for asbestos exposure and lifetime smoking (pack-years), the odds ratio was 1.41 (95% CI = 1.06, 1.88). Both crude odds ratio and asbestos exposure as well as lifetime smoking adjusted odds ratio for the ≥65 years of age group were not significant. Increasing years of diesel exhaust exposure, categorized as ≥20 diesel years and 5 to 19 diesel years, with 0 to 4 years as the referent group, showed significantly increased risk in the ≤64 years of age group after adjusting for asbestos exposure and pack-year category of smoking. For individuals who had ≥20 years of diesel exhaust exposure, the odds ratio was 1.64 (95% CI = 1.18, 2.29), whereas among individuals who had 5 to 19 years of diesel exhaust exposure, the odds ratio was 1.02 (95% CI = 0.72, 1.45). In the ≥65 years of age group, only 3% of the workers were exposed to diesel exhaust for more than 20 years. Relative odds for 5 to 19 years and ≥20 years of diesel exposure were less than 1 (p>0.01) after adjusting for smoking and asbestos exposure.

Alternate models to explain post-asbestos exposure were tested. These were variables for regular and intermittent exposure groups and an estimate of years of exposure based on estimated years worked prior to 1959. No differences in results were seen. The interactions between diesel exhaust exposure and the three pack-year categories (<50, >50, and missing pack-years) were explored. The cross-product terms were not significant. A model was also tested that excluded recent diesel exhaust exposure occurring within the 5 years before death and gave an odds ratio of 1.43 (95% CI = 1.06, 1.94) adjusted for cigarette smoking and asbestos exposure, for workers with 15 years of cumulative exposure. For workers with 5 to 14 years of cumulative exposure, the relative odds were not significant.

The many strengths of the study are consideration of confounding factors such as asbestos exposure and smoking; classification of diesel exhaust exposures by job titles and industrial hygiene sampling; exploration of interactions between smoking, asbestos exposure, and diesel exhaust exposure; and good ascertainment (87%) of death certificates from the 15,059 deaths reported by the RRB.

The investigators also recognized and reported the following limitations: overestimation of cigarette consumption by surrogate respondents, which may have exaggerated the contribution of smoking to lung cancer risk, and use of the Interstate Commerce Commission (ICC) job classification as a surrogate for exposure, which may have led to misclassification of diesel exhaust exposure jobs with low intensity and intermittent exposure, such as railroad police and bus drivers, as unexposed. These two limitations would result in the underestimation of the lung cancer risk. This source of error could have been avoided if diesel exhaust exposures were categorized by a specific dose range associated with a job title that could have been classified as heavy, medium, low, and zero exposure instead of a dichotomous variable. The use of death certificates to identify cases and controls may have resulted in misclassification. Controls may have had undiagnosed primary lung cancer, and lung cancer cases might have been secondary lesions misdiagnosed as primary lung cancer. However, the investigators quote a third National Cancer Survey report in which the death certificates for lung cancer were coded appropriately in 95% of the cases. Last, as in all previous studies, there is a lack of data on the contribution of unknown occupational or environmental exposures and passive smoking. In conclusion, this study, compared with previous studies (on diesel exposure and lung cancer risk), provides the most valid evidence that occupational diesel exhaust emission exposure increases the risk of lung cancer.

7.2.2.6. *Benhamou et al. (1988): Occupational Risk Factors of Lung Cancer in a French Case-Control Study*

This is a case-control study of 1,625 histologically confirmed cases of lung cancer and 3,091 matched controls, conducted in France between 1976 and 1980. This study was part of an international study to investigate the role of smoking and lung cancer. Each case was matched with one or two controls whose diseases were not related to tobacco use, sex, age at diagnosis (±5 years), hospital of admission, or interviewer. Information was obtained from both cases and controls on place of residence since birth, educational level, smoking, and drinking habits. A complete lifetime occupational history was obtained by asking participants to give their occupations from the most recent to the first. Women were excluded because most of them had listed no occupation. Men who smoked cigars and pipes were excluded because there were very

few in this category. Thus, the study was restricted to nonsmokers and cigarette smokers. Cigarette smoking exposure was defined by age at the first cigarette (nonsmokers, ≤20 years, or >20 years), daily consumption of cigarettes (nonsmokers, <20 cigarettes a day, and ≥20 cigarettes a day), and duration of cigarette smoking (nonsmokers, <35 years, and ≥35 years). The data on occupations were coded by a panel of experts according to their own chemical or physical exposure determinations. Occupations were recorded blindly using the International Standard Classification of Occupations. Data on 1,260 cases and 2,084 controls were available for analysis. The remaining 365 cases and 1,007 controls were excluded because they did not satisfy the required smoking status criteria.

A matched logistic regression analysis was performed to estimate the effect of each occupational exposure after adjusting for cigarette status. Matched relative risk ratios were calculated for each occupation with the baseline category, which consisted of patients who had never been engaged in that particular occupation. The matched relative risk ratios adjusted for cigarette smoking for the major groups of occupations showed that the risks were significantly higher for production and related workers, transport equipment operators, and laborers (RR = 1.24, 95% CI = 1.04, 1.47). On further analysis of this group, for occupations with potential diesel emission exposure, significant excess risks were found for motor vehicle drivers (RR = 1.42, 95% CI = 1.07, 1.89) and transport equipment operators (RR = 1.35, 95% CI = 1.05, 1.75). No interaction with smoking status was found in any of the occupations. The only other significant excess was observed for miners and quarrymen (RR = 2.14, 95% CI = 1.07, 4.31). None of the significant associations showed a dose-response relationship with duration of exposure.

This study was designed primarily to investigate the relationship between smoking (not occupations or environmental exposures) and lung cancer. Although an attempt was made to obtain complete occupational histories, the authors did not clarify whether, in the logistic regression analysis, they used the subjects' first occupation, predominant occupation, last occupation, or ever worked in that occupation as the risk factor of interest. The most important limitation of this study is that the occupations were not coded into exposures for different chemical and physical agents, thus precluding the calculation of relative risks for diesel exposure. Using occupations as surrogate measures of diesel exposure, an excess significant risk was obtained for motor vehicle drivers and transport equipment operators, but not for motor mechanics. However, it is not known if subjects in these occupations worked with diesel engines or nondiesel engines.

7.2.2.7. *Hayes et al. (1989): Lung Cancer in Motor Exhaust-Related Occupations*

This study reports the findings from an analysis of pooled data from three lung cancer case-control studies that examine in detail the association between employment in motor exhaust-related (MER) occupations and lung cancer risk adjusted for confounding by smoking and other risk factors. The three studies were carried out by the National Cancer Institute in Florida (1976 to 1979), New Jersey (1980 to 1981), and Louisiana (1979 to 1983). These three studies were selected because the combined group would provide a sufficient sample to detect a risk of lung cancer in excess of 50% among workers in MER occupations. The analyses were restricted to males who had given occupational history. The Florida study was hospital based, with cases ascertained through death certificates. Controls were randomly selected from hospital records and death certificates, excluding psychiatric diseases, matched by age and county. The New Jersey study was population based, with cases ascertained through hospital records, cancer registry, and death certificates. Controls were selected from among the pool of New Jersey licensed drivers and death certificates. The Louisiana study was hospital based (it is not specified how the cases were ascertained), and controls were randomly selected from hospital patients, excluding those with lung diseases and tobacco-related cancers.

A total of 2,291 cases of male lung cancers and 2,570 controls were eligible, and the data on occupations were collected by next-of-kin interviews for all jobs held for 6 months or more, including the industry, occupation, and number of years employed. The proportion of next-of-kin interviews varied by site from 50% in Louisiana to 85% in Florida. The coding schemes were reviewed to identify MER occupations, which included truck drivers and heavy equipment operators (cranes, bulldozers, and graders); bus drivers, taxi drivers, chauffeurs, and other motor vehicle drivers; and automobile and truck mechanics. Truck drivers were classified as routemen and delivery men and other truck drivers. All jobs were also classified with respect to potential exposure to known and suspected lung carcinogens. Odds ratios were calculated by the maximum likelihood method adjusting for age by birth year, usual amount smoked, and study area. Logistic regression models were used to examine the interrelationship of multiple variables.

A statistically significant excess risk was detected for employment of 10 years or more for all MER occupations (except truck drivers) adjusted for birth cohort, usual daily cigarette use, and study area. The odds ratio for lung cancer using data gathered by direct interviews was 1.4 (95% CI = 1.1, 2.0), allowing for multiple MER employment, and 2.0 (95% CI = 1.3, 3.0), excluding individuals with multiple MER employment. Odds ratios for all MER employment, except truck drivers who were employed for less than 10 years, were 1.3 (95% CI = 1.0, 1.7) and 1.3 (95% CI = 0.9, 1.8) including and excluding multiple MER employment, respectively. Odds ratios were then derived for specific MER occupations and, to avoid the confounding effects of

multiple MER job classifications, analyses were also done excluding subjects with multiple MER job exposures. Truck drivers employed for more than 10 years had an odds ratio of 1.5 (95% CI = 1.1, 1.9). A similar figure was obtained excluding subjects with multiple MER employment. An excess risk was not detected for truck drivers employed less than 10 years. The only other job category that showed a statistically significant excess for lung cancer was the one that included taxi drivers and chauffeurs who worked multiple MER jobs for less than 10 years (OR = 2.5, 95% CI = 1.4, 4.8). For the same category, the risk for individuals working in that job for more than 10 years was 1.2 (95% CI = 0.5, 2.6). A statistical significant positive trend (p<0.05) with increasing employment of <2 years, 2 to 9 years, 10 to 19 years, and 20+ years was observed for truck drivers but not for other MER occupations. A statistically nonsignificant excess risk was also observed for heavy equipment operators, bus drivers, taxi drivers and chauffeurs, and mechanics employed for 10 years or more. All of the above-mentioned odds ratios were derived, adjusted for birth cohort, usual daily cigarette use, and State of residence. Exposure to other occupational suspect lung carcinogens did not account for the excess risks detected.

Results of this large study provide evidence that workers in MER jobs are at an excess risk of lung cancer that is not explained by their smoking habits or exposures to other lung cancers. Because no information on type of engine had been collected, it was not possible to determine if the excess risk was due to exposure to diesel exhaust or gasoline exhaust or the mixture of the two. Among the study's limitations are possible bias due to misclassification of jobs reported by the large proportion of next-of-kin interviews and the problems in classifying individuals into uniform occupational groups based on the pooled data in the three studies that used different occupational classification schemes.

7.2.2.8. *Steenland et al. (1990): A Case-Control Study of Lung Cancer and Truck Driving in the Teamsters Union*

Steenland et al. conducted a case-control study of lung cancer deaths in the Teamsters Union to determine the risk of lung cancer among different occupations. Death certificates were obtained from the Teamsters Union files in the central States for 10,485 (98%) male decedents who had filed claims for pension benefits and who had died in 1982 and 1983. Individuals were required to have 20 years' tenure in the union to be eligible to claim benefits. Cases comprised all deaths (n = 1,288) from lung cancer, coded as ICD 162 or 163 for underlying or contributory cause on the death certificate. The 1,452 controls comprised every sixth death from the entire file, excluding deaths from lung cancer, bladder cancer, and motor vehicle accidents. Detailed information on work history and potential confounders such as smoking, diet, and asbestos exposure was obtained by questionnaire. Seventy-six percent of the interviews were provided by

spouses and the remainder by some other next of kin. The response rate was 82% for cases and 80% for controls. Using these interview data and the 1980 census occupation and industry codes, subjects were classified either as nonexposed or as having held other jobs with potential diesel exhaust exposure. Data on job categories were missing for 12% of the study subjects. A second work history file was also created based on the Teamsters Union pension application that lists occupation, employer, and dates of employment. A three-digit U.S. census code for occupation and industry was assigned to each job for each individual. This Teamsters Union work history file did not have information on whether men drove diesel or gasoline trucks, and the four principal occupations were long-haul drivers, short-haul or city drivers, truck mechanics, and dockworkers. Subjects were assigned the job category in which they had worked the longest.

The case-control analysis was done using unconditional logistic regression. Separate analyses were conducted for work histories from the Teamsters Union pension file and from next-of-kin interviews. Covariate data were obtained from next-of-kin interviews. Analyses were also performed for two time periods: employment after 1959 and employment after 1964. These two cut-off years reflect years of presumed dieselization; 1960 for most trucking companies and 1965 for independent driver and nontrucking firms. Data for analysis could be obtained for 994 cases and 1,085 controls using Teamsters Union work history and for 872 cases and 957 controls using next-of-kin work history. When exposure was considered as a dichotomous variable, for both Teamsters Union and next-of-kin work history, no single job category had an elevated risk. From the next-of-kin data, diesel truck drivers had an odds ratio of 1.42 (95% CI = 0.74, 2.47) and diesel truck mechanics had an odds ratio of 1.35 (95% CI = 0.74, 2.47). Odds ratios by duration of employment as a categorical variable were then estimated. For the Teamsters Union work history data and when only employment after 1959 was considered, both long-haul (p<0.04) and short-haul drivers (not significant) showed an increase in risk with increased years of exposure. The length of employment categories for which the trends were analyzed were 1 to 11 years, 12 to 17 years, and 18 years or more. Using 1964 as the cutoff date, long-haul drivers continued to show a significant positive trend (p=0.04), with an odds ratio of 1.64 (95% CI = 1.05, 2.57) for those who worked for 13+ years, the highest category. Short-haul drivers, however, did not show a positive trend when 1964 was used as the cutoff date. Similar trend analysis was done for most next-of-kin data. A marginal increase in risk with increasing duration of employment as a truck driver (p=0.12) was observed. For truck drivers who primarily drove diesel trucks for 35 years or longer, the odds ratio for lung cancer was 1.89 (95% CI = 1.04, 3.42). The odds ratio was 1.34 (95% CI = 0.81, 2.22) for gasoline truck drivers and 1.09 (95% CI = 0.44, 2.66) for truck mechanics. No significant interactions between age and diesel exhaust exposure or smoking

and diesel exhaust exposure were observed. All the odds ratios were adjusted for age, smoking, and asbestos in addition to various exposure categories.

The authors acknowledge several limitations of this study, which include possible misclassifications of exposure and smoking habits, as information was provided by next of kin; lack of sufficient latency to observe lung cancer excess; and a small nonexposed group (n = 120). Also, concordance between Teamsters Union and next-of-kin job categories could not be easily evaluated because job categories were defined differently in each data set. No data were available on levels of diesel exposure for the different job categories. Given these limitations, the positive findings of this study are probably underestimated.

7.2.2.9. *Steenland et al. (1998): Diesel Exhaust and Lung Cancer in the Trucking Industry: Exposure-Response Analyses and Risk Assessment*

Steenland et al. (1998) conducted an exposure-response analysis by supplementing the data from their earlier case-control study of lung cancer and truck drivers in the Teamsters Union (Steenland et al., 1990) with exposure estimates based on a 1990 industrial hygiene survey of elemental carbon exposures a surrogate for diesel exhaust in the trucking industry.

Study subjects were long-term Teamsters enrolled in the pension system who died during the period 1982-1983. Using death certificate information, the researchers identified 994 cases of lung cancer for the study period, and 1,085 non lung cancer deaths served as controls. Subjects were divided into job categories based on the job each held the longest. Most had held only one type of job. The job categories were short-haul driver, long-haul driver, mechanic, dockworker, other jobs with potential diesel exposure, and jobs outside the trucking industry without occupational diesel exposure. Smoking histories were obtained from next of kin. Odds ratios were calculated for work in an exposed job category at any time and after 1959 (an estimated date when the majority of heavy duty trucks had converted to diesel) compared with work in nonexposed jobs. Odds ratios were adjusted for age, smoking, and potential asbestos exposure. Trends in effect estimates for duration of work in an exposed job were also calculated. An industrial hygiene survey by Zaebst et al. (1991) of elemental carbon exposures in the trucking industry provided exposure estimates for each job category in 1990. The elemental carbon measurements were generally consistent with the epidemiologic results, in that mechanics are found to have the highest exposures and relative risk, followed by long-haul and then short-haul drivers, although dockworkers have the highest exposures and the lowest relative risks.

Past exposures were estimated assuming that they were a function of (1) the number of heavy-duty trucks on the road, (2) the particulate emissions (grams/mile) of diesel engines over time, and (3) leaks from truck exhaust systems for long-haul drivers. Estimates of past exposure

to elemental carbon, as a marker for diesel exhaust exposure, for subjects in the case-control study were made by assuming that average 1990 levels for a job category could be assigned to all subjects in that category, and that levels prior to 1990 were directly proportional to vehicle miles traveled by heavy-duty trucks and the estimated emission levels of diesel engines. A 1975 exposure level of elemental carbon in term of micrograms per cubic meter was estimated by the following equation: 1975 level = 1990 level*(vehicle miles 1975/vehicle miles 1990) (emissions 1975/emissions 1990). Once estimates of exposure for each year of work history were derived for each subject, analyses were conducted by cumulative level of estimated carbon exposure.

Estimates were made for long-haul drivers (n = 1,237), short-haul drivers (n = 297), dockworkers (n = 164), mechanics (n = 88), and those outside the trucking industry (n = 150). Logistic regression was used to estimate odds ratios adjusted for five categories of age, race, smoking (never, former-quitting before 1963, former-quitting in 1963 or later, current-with <1 pack per day, and current-with 1 or more packs per day), diet, and reported asbestos exposure. A variety of models for cumulative exposure were considered, including a log-linear model with cumulative exposure, a model adding a quadratic term for cumulative exposure, a log transform of cumulative exposure, dummy variables for quartile of cumulative exposure, and smoothing splines of cumulative exposure. The estimates of rate ratios from logistic regression for specific levels of exposure to elemental carbon were then used to derive excess risk estimates for lung cancer after lifetime exposure to elemental carbon.

The log of cumulative exposure was found to be the best fitting model and was a significant predictor ($p = 0.01$). Odds ratios for quartile of cumulative exposure show a pattern of significantly increasing trends in risk with increasing exposure, ranging between 1.08 and 1.72, depending on the exposure level and lag structure used. The lifetime excess risk of lung cancer death (through age 75) for a male truck driver was estimated to be in the range of 1.4-2.3% (95% confidence limits ranged from 0.3% to 4.6%) above the background risk, depending on the emissions scenarios assumed. The authors conclude that the data suggest a positive and significant increase in lung cancer risk with increasing estimated cumulative exposure to diesel exhaust among workers in the trucking industry. They assert that these estimates suggest that the lifetime excess risk for lung cancer is 10 times higher than the OSHA standards, but caution that the results should be viewed as exploratory.

The authors acknowledge that the increasing trend in risk with increasing estimates of cumulative exposure is partly due to the fact that a component of cumulative dose is simple duration of exposure, and that analyses by simple duration also exhibit a positive trend with duration. This analysis essentially weights the duration by contrived estimates of exposure intensity, and they acknowledge that this weighting depends on very broad assumptions.

This is not an analysis of new data that provides independent estimates of relative risk for diesel exhaust and lung cancer incidence. Instead, it is an attempt to convert the data from Steenland's earlier study of lung cancer for the purpose of estimating a different risk metric, "lifetime excess risk of lung cancer," by augmenting these data with limited industrial hygiene data and rationalizations about plausible models for cumulative exposure.

The Health Effects Institute (HEI, 1999) and others have raised some questions about the exposure estimations and control for confounding variables. EPA and NIOSH will address these concerns in the year 2000. It should be noted that these concerns are about the use of these data for quantitative risk assessment. As far as qualitative risk assessment is concerned, this study is still considered to be positive and strong.

7.2.2.10. *Boffetta et al. (1990): Case-Control Study on Occupational Exposure to Diesel Exhaust and Lung Cancer Risk*

This is an ongoing (since 1969) case-control study of tobacco-related diseases in 18 hospitals (six U.S. cities). Cases comprise 2,584 males with histologically confirmed primary lung cancers. Sixty-nine cases were matched to 1 control, whereas 2,515 were matched to 2 controls. Controls were individuals who were diagnosed with non-tobacco-related diseases. The matching was done for sex, age (±2 years), hospital, and year of interview. The interviews were conducted at the hospitals at the time of diagnosis. In 1985, the occupational section of the questionnaire was modified to include the usual occupation and up to five other jobs as well as duration (in years) worked in those jobs. After 1985, information was also obtained on exposure to 45 groups of chemicals, including diesel exhaust at the workplace or during hobby activities. A priori aggregation of occupations was categorized into low probability of diesel exhaust exposure (reference group), possible exposure (19 occupations), and probable exposure (13 occupations). Analysis was conducted based on "usual occupation" on all study subjects, and any occupation with sufficient cases was eligible for further analysis. In addition, cases enrolled after 1985 for which there were self-reported diesel exhaust exposure and detailed work histories were also analyzed separately.

Both matched and unmatched analyses were done by calculating the adjusted (for smoking and education) relative odds using the Mantel-Haenzael method and calculating the test-based 95% confidence interval using the Miettinen method. Unconditional logistic regression was used to adjust for potential confounders (the PROC LOGIST of SAS). Linear trends for risk were also tested according to Mantel.

Adjusted relative odds for possible and probable exposure groups as well as the truck drivers were slightly below unity, none being statistically significant for the entire study

population. Although slight excesses were observed for the self-reported diesel exhaust exposure group and the subset of post-1985 enrollees for highest duration of exposure (for self-reported exposure, occupations with probable exposure and for truck drivers), none was statistically significant. Trend tests for the risk of lung cancer among self-reported diesel exhaust exposure, probable exposure, and truck drivers with increasing exposure (duration of exposure used as surrogate for increasing dose) were nonsignificant too. Statistically significant lung cancer excesses were observed for cigarette smoking only.

The major strength of this study is availability of detailed smoking history. Even though detailed information was obtained for the usual and five other occupations (1985), no effort was made to estimate or verify the actual exposure to diesel exhaust; instead, duration of employment was used as a surrogate for dose. The numbers of cases and controls were large; however, the number of individuals exposed to diesel exhaust was relatively few, thus reducing the power of the study. This study did not attempt latency analysis either. Given these limitations, the findings of this study are unable to provide either positive or negative evidence for a causal association between diesel exhaust and occurrence of lung cancer.

7.2.2.11. *Emmelin et al. (1993): Diesel Exhaust Exposure and Smoking: A Case-Referent Study of Lung Cancer Among Swedish Dock Workers*

This is a case-control study of lung cancer drawn from the cohort defined as all-male workers who had been employed as dockworkers for at least 6 months between 1950 and 1974. In the population of 6,573 from 20 ports, there were 90 lung cancer deaths (cases), identified through Swedish death and cancer registers, during the period 1960 to 1982. Of these 90 deaths, the 54 who were workers at the 15 ports for which exposure surrogate information was available were chosen for the case-control study. Four controls, matched on port and age, were chosen for each case from the remaining cohort who had survived to the time of diagnosis of the case. Both live and deceased controls were included. The final analyses were done on 50 cases and 154 controls who had complete information on employment dates and smoking data. The smoking strata were created by classifying ex-smokers as nonsmokers if they had not smoked for at least 5 years prior to the date of diagnosis of the case; otherwise they were classified as smokers.

Relative odds and regression coefficients were calculated using conditional logistic regression models. Comparisons were made both with and without smoking included as a variable, and the possible interaction between smoking and diesel exhaust was tested. Both the weighted linear regressions of the adjusted relative odds and the regression coefficients were used to test mortality trends with all three exposure variables.

Exposure to diesel exhaust was assessed indirectly by initially measuring (1) exposure intensity based on exhaust emission, (2) characteristics of the environment in terms of ventilation, and (3) measures of proportion of time in higher exposed jobs. For exhaust emissions, annual diesel fuel consumption at a port was used as the surrogate. For ventilation, the annual proportion of ships with closed or semiclosed holds was used as the surrogate. The proportion of time spent below decks was used as the surrogate for more exposed jobs. Although data were collected for all three measures, only the annual fuel consumption was used for analysis. Because every man was likely to rotate through the various jobs, the authors thought using annual consumption of diesel fuel was the appropriate measure of exposure. Consequently, in a second analysis, the annual fuel consumption was divided by the number of employees in the same port that year to come up with the fuel-per-person measure, which was further used to create a second measure, "exposed time." The "annual fuel" and exposed-time data were entered in a calendar time-exposure matrix for each port, from which individual exposure measures were created. A third measure, "machine time" (years of employment from first exposure), was also used to compare the results with other studies. All exposure measures were accumulated from the first year of employment or first year of diesel machine use, whichever came later. The last year of exposure was fixed at 1979. All exposures up to 2 years before the date of lung cancer diagnosis were omitted from both cases and matched controls. A priori classification into three categories of low, medium, and high exposure was done for all three exposure variables: machine time, fuel, and exposed time.

Conditional logistic regression models, adjusting for smoking status and using low exposures and/or nonsmokers as a comparison group, yielded positive trends for all exposure measures, but no trend test results were reported, and only the relative odds for the exposed-time exposure measure in the high-exposure group (OR = 6.8, 90% CI = 1.3 to 34.9) was reported as statistically significant. For smokers, adjusting for diesel exhaust exposure level, the relative odds were statistically significant and about equal for all three exposure variables: machine time, OR = 5.7 (90% CI = 2.4 to 13.3); fuel, OR = 5.5 (90% CI = 2.4 to 12.7); and exposed time, OR = 6.2 (90% CI = 2.6 to 14.6). Interaction between diesel exhaust and smoking was tested by conditional logistic regression in the exposed-time variable. Although there were positive trends for both smokers and nonsmokers, the trend for smokers was much steeper: low, OR = 3.7 (90% CI = 0.9 to 14.6); medium, OR = 10.7 (90% CI = 1.5 to 78.4); and high, OR = 28.9 (90% CI = 3.5 to 240) indicating more than additive interaction between these two variables.

In the weighted linear regression model with the exposed-time variable, the results were similar to those using the logistic regression model. The authors also explored the smoking variable further in various analyses, some of which suggested a strong interaction between diesel

exhaust and smoking. However, with just six nonsmokers and no further categorization of smoking amount or duration, these results are of limited value.

The diesel exhaust exposure matrices created using three different variables are intricate. Analyses by any of these variables essentially yield the same positive results and positive trends, providing consistent support for a real effect of diesel exhaust exposure, at least in smokers. However, methodological limitations to this study prevent a more definitive conclusion. The numbers of cases and controls are small. There are very few nonsmokers; thus testing the effects of diesel exhaust exposure in them is futile. Lack of information on asbestos exposure, to which dockworkers are usually exposed, may also confound the results. Also, no latency analyses are presented. Overall, despite these limitations, this study supports the earlier findings of excess lung cancer mortality among individuals exposed to diesel exhaust.

Table 7-2 summarizes the above lung cancer case-control studies.

7.2.3. Case-Control Study of Prostate Cancer

7.2.3.1. *Aronsen et al. (1996): Occupational Risk Factors for Prostate Cancer: Results from a Case-Control Study in Montreal, Quebec, Canada*

A population-based case-control study was undertaken in 1979 to explore possible associations between many types of cancer and hundreds of occupational exposures. The current report provides a more refined analysis focusing only on prostate cancer and those exposures that showed associations with this site in the original analysis.

A total of 557 cases of incident, histologically confirmed prostate cancer were identified among males aged 35 to 70 years resident in the Montreal area. The timeframe for eligibility of incident cancers was not provided. Of these, 449 (81%) subjects were interviewed. Two sets of controls were used. Out of 740 population controls, 533 (72%) persons identified by random digit dialing or electoral lists provided interview data. Additionally, 1,550 controls were selected from the non–prostate cancer cases identified in the original study. Both control groups were pooled for the analysis after determining that the estimates did not depend on the control group.

The exposure data were obtained by interview questionnaire, with a structured section requesting information on potential confounders and a semistructured probing section designed to obtain a detailed description of each job the subject had in his working lifetime. A team of chemists and hygienists translated each job into a list of potential exposures by means of a checklist of 294 substances. The current analysis focused on 17 occupations, 11 industries, and

Table 7-2. Epidemiologic studies of the health effects of exposure to diesel exhaust: case-control studies of lung cancer

Authors	Population studied	Diesel exhaust exposure	Results	Limitations
Williams et al. (1977)	7,518 (3,539 males and 3,979 females) incident invasive cancers from the Third National Cancer Survey Lung cancer cases: 32 in males 28 in females Combined other cancer sites were used as controls	Main lifetime, recent, and other employment information obtained at the time of survey 1970 Census Coding Scheme for Employment was used to code the occupations by one of the authors	SNS elevated relative odds were observed among occupations of trucking, railroading, and mining	Exposure estimation based on self-report that was not validated 47% nonresponse Control group consisted of other cancers, probably diluting the risk estimation Small numbers in cause-specific cancers and individual occupations
Hall and Wynder (1984)	502 histologically confirmed lung cancers Cases diagnosed 12 mo prior to interviews 502 matched hospital controls without tobacco-related diseases, matched for age, sex, race, and geographical area Population from 18 hospitals in controls	Based on previous Industrial Hygiene Standards for a particular occupation, usual lifetime occupation coded as “probably high exposure” and “no exposure” NIOSH standards used to classify exposures: High Moderate Low	SNS excess risk after adjustment for smoking for lung cancer: RR = 1.4 (1st criteria) and RR = 1.7 (NIOSH criteria)	Complete lifetime employment history not available Self-reported occupation history not validated No analysis by dose, latency, or duration of exposure No information on nonoccupational diesel exposure

Table 7-2. Epidemiologic studies of the health effects of exposure to diesel exhaust: case-control studies of lung cancer

Authors	Population studied	Diesel exhaust exposure	Results	Limitations
Damber and Larsson (1987)	589 lung cancer cases who had died prior to 1979 reported to Swedish registry between 1972 and 1977 582 matched dead controls (sex, age, year of death, municipality) drawn from National Registry of Cause of Death 453 matched living controls (sex, year of birth, municipality) drawn from National Population Registry	Occupations held for at least 1 year or more Using a 5-digit code the occupations were classified according to Nordic Classification of Occupations	SS OR = 2.7 (≥1 year of employment) SS OR = 9.8 (≥20 years of employment) Adjustment for smoking was done SNS OR = 1.2 for professional drivers (≥20 years of employment) with dead controls SNS OR = 1.1 ≥20 years of employment) with living controls	Uncertain diesel exhaust exposure No validation of exposure done Underground miners data not adjusted for other confounders such as radon, etc.
Lerchen et al. (1987)	506 lung cancer cases from New Mexico tumor registry (333 males and 173 females) Aged 25-84 years Diagnosed between January 1, 1980, and December 31, 1982 771 (499 males and 272 females) frequency matched with cases, selected from telephone directory	Lifetime occupational history and self-reported exposure history were obtained Coded according to Standard Industrial Classification Scheme	No excess of relative odds was observed for diesel exhaust exposure	Exposure based on occupational history and self-report, which was not validated 50% occupational history provided by next of kin Absence of lung cancer association with asbestos suggests misclassification of exposure
Garshick et al. (1987)	1,319 lung cancer cases who died between March 1, 1981, and February 28, 1982 2,385 matched controls (two each, age and date of death) Both cases and controls drawn from railroad worker cohort who had worked for 10 or more years	Personal exposure assessed for 39 job categories This was corrected with job titles to dichotomize the exposure into: Exposed Not exposed	SS OR = 1.41 (≤64 year age group) SS OR = 1.64 (≤64 year age group) for ≥20 years diesel exhaust exposure group when compared to 0- to 4-year exposure group All ORs adjusted for lifetime smoking and asbestos exposure	Probable misclassification of diesel exhaust exposure jobs Years of exposure used as surrogate for dose 13% of death certificates not ascertained Overestimation of smoking history

Table 7-2. Epidemiologic studies of the health effects of exposure to diesel exhaust: case-control studies of lung cancer

Authors	Population studied	Diesel exhaust exposure	Results	Limitations
Benhamou et al. (1988)	1,260 histologically confirmed lung cancer cases 2,084 non-tobacco-related disease matched controls (sex, age at diagnosis, hospital admission, and interviewer) Occurring between 1976 and 1980 in France	Based on exposures determined by panel of experts The occupations were recorded blindly using International Standard Classification of Occupations as chemical or physical exposures	Significant excess risks were found in motor vehicle drivers (RR = 1.42) and transport equipment operators (RR = 1.35) (smoking adjusted)	Exposure based on occupational histories not validated Exposures classified as chemical and physical exposure, not specific to diesel exhaust
Hayes et al. (1989)	Pooled data from three different studies consisting of 2,291 male lung cancer cases 2,570 controls	Occupational information from next of kin for all jobs held Jobs classified with respect to potential exposure to known and suspected pulmonary carcinogens	SS OR = 1.5 for truck drivers (>10 years of employment) SS positive trend with increasing employment as truck driver	Exposure data based on job description given by next of kin, which was not validated Could have been mixed exposure to both diesel and gasoline exhausts Job description could have led to misclassification

Table 7-2. Epidemiologic studies of the health effects of exposure to diesel exhaust: case-control studies of lung cancer

Authors	Population studied	Diesel exhaust exposure	Results	Limitations
Steenland et al. (1990)	1,058 male lung cancer deaths between 1982 and 1983 1,160 every sixth death from entire mortality file sorted by social security number (excluding lung cancer, bladder cancer, and motor vehicle accidents) Cases and controls were from Central State Teamsters who had filed claims (requiring 20-year tenure).	Longest job held: diesel truck driver, gasoline truck driver, both types of trucks, truck mechanic, and dockworkers	As 1964 cut-off point: SS OR = 1.64 for long-haul drivers with 13+ years of employment Positive trend test for long-haul drivers (p=0.04) SS OR = 1.89 for diesel truck drivers of 35+ years of employment	Exposure based on job titles not validated Possible misclassification of exposure and smoking, based on next-of-kin information Lack of sufficient latency
Boffetta et al. (1990)	From 18 hospitals (since 1969) 2,584 male lung cancer cases matched to either one control (69) or two controls (2,515) were drawn. Matched on age, hospital, and year of interview	A priori aggregation of occupations categorized into low probability, possible exposure (19 occupations), and probable exposure (13 occupations) to diesel exhaust	OR slightly below unity SNS	No verification of exposure Duration of employment used as surrogate for dose Number of individuals exposed to diesel exhaust was small
Emmelin et al. (1993)	50 male lung cancer cases from 15 ports (worked for at least 6 months between 1950 and 1974), 154 controls matched on age and port	Indirect diesel exhaust exposure assessment done based on (1) exposure intensity, (2) characteristics of ventilation, (3) measure of proportion of time in higher exposure jobs	SS OR for high-exposure group = 6.8	Numbers of cases and controls are small Very few nonsmokers Lack of exposure information on asbestos No latency analysis

Abbreviations: OR = odds ratio; RR = relative risk; SNS = statistically nonsignificant; SS = statistically significant.

27 substances as exposures. Unconditional logistic regression models were used to provide effect estimates adjusted for potential confounding by nonoccupational variables such as age, family income, ethnicity, Quetelet index, and respondent status. Diesel exhaust exposure was identified by reporting a history of work as a truck driver or as a heavy machinery operator.

The odds ratio for "possible exposure" to diesel exhaust is 1.47 (95% CI = 1.01, 2.13). When the criteria for exposure were "substantial" (defined by rating both Concentration and frequency of exposure as medium or high), the odds ratio is 1.10 (95% CI= 0.72, 1.67). Diesel exhaust showed an increase in risk with duration of exposure (1-10 vs. 11+ years). The odds ratio for 11 or more years of exposure is 1.5 (95% CI = 1.1, 2.1). Risk is not positively correlated with concentration (low vs. medium/high) or frequency of exposure (low/medium vs. high).

This study identifies associations between diesel exhaust exposure as inferred by occupational history and histologically confirmed incident prostate cancers. The crude exposure assessment and lack of a substantive a priori hypothesis for the relation require that the results be considered preliminary or exploratory. The lack of control for smoking and diminished effect with increasing certainty of exposure also undermine the credibility of the observed association.

7.2.4. Summaries of Studies and Meta-Analyses of Lung Cancer

7.2.4.1. *Cohen and Higgins (1995): Health Effects of Diesel Exhaust: Epidemiology*

The Health Effects Institute (HEI) reviewed all published epidemiologic studies on the health effects of exposure to diesel exhaust available through June 1993 identified by a MEDLINE search and by reviewing the reference sections of published research and earlier reviews. HEI identified 35 reports of epidemiologic studies (16 cohort and 19 case-control) of the relation of occupational exposure to diesel emissions and lung cancer published between 1957 and 1993.

HEI reviewed the 35 reports for epidemiologic evidence of health effects of exposure to diesel exhaust for lung cancer, other cancers, and nonmalignant respiratory disease. They found that the data were strongest for lung cancer. The evidence suggested that occupational exposure to diesel exhaust from diverse sources increases the rate of lung cancer by 20% to 40% in exposed workers generally, and to a greater extent among workers with prolonged exposure. They also found that the results are not explicable by confounding caused by cigarette smoking or other known sources of bias.

Control for smoking was identified in 15 studies. Six studies (17%) reported relative risk estimates less than one; 29 studies (83%) reported at least relative risk indicating positive association. Twelve studies indicating a relative risk greater than 1 had 95% confidence intervals, which excluded unity.

The authors conclude that epidemiologic data consistently show weak associations between exposure to diesel exhaust and lung cancer. They find that the evidence suggests that long-term exposure to diesel exhaust in a variety of occupational circumstances is associated with a 1.2- to 1.5-fold increase in the relative risk of lung cancer compared with workers classified as unexposed. Most of the studies that controlled for smoking found that the association between increased risk of lung cancer and exposure to diesel exhaust persisted after such controls were applied, although in some cases the excess risk was lower. None of the studies measured exposure to diesel emissions or characterized the actual emissions from the source of exposure for the time period most relevant to the development of lung cancer. Most investigators classified exposure on the basis of work histories reported by subjects or their next of kin, or by retirement records. Although these data provide relative rankings of exposure, the absence of concurrent exposure information is the key factor that limits interpretation of the epidemiologic findings and subsequently their utility in making quantitative estimates of cancer risks.

This is a comprehensive and thorough narrative review of studies of the health effects of diesel exhaust. It does not undertake formal estimation of summary measures of effect or evaluation of heterogeneity in the results. The conclusion drawn about the consistency of the results is based on the author's assessment of the failure of potential biases and alternative explanations for the increase in risk to account for the observed consistency. In many if not most studies, the quality of the data used to control confounding was relatively crude. Although the studies do include qualitative assessment of whether control for smoking is taken into account, careful scrutiny of the quality of the control or adjustment for smoking among the studies is absent. This leaves open the possibility that prevalent residual confounding by inadequate control for smoking in many or most studies may account for the consistent associations seen.

7.2.4.2. *Bhatia et al. (1998): Diesel Exhaust Exposure and Lung Cancer*

Bhatia et al. (1998) report a meta-analysis of 29 published cohort and case-control studies of the relation between occupational exposure to diesel exhaust and lung cancer. A search of the epidemiologic literature was conducted for all studies concerning lung cancer and diesel exhaust exposure. Occupational studies involving mining were excluded because of concern about the possible influence of radon and silica exposures. Studies in which the minimum interval from time of first exposure to end of followup was less than 10 years, and studies in which work with diesel equipment or engines could not be confirmed or reliably inferred, were excluded. When studies presented risk estimates for more than one specific occupational category of diesel exhaust-exposed workers, the subgroup risk estimates were used in the meta-analysis. Smoking-adjusted effect measures were used when present.

Thirty-five studies were identified in the literature search, of which 23 met the criteria for inclusion in the meta-analysis. The observed relative risk estimates were greater than 1 in 21 of these studies; this result is unlikely to be due to chance. The pooled relative risk weighted by study precision was 1.33 (95% CI = 1.24, 1.44) indicated increased relative risk for lung cancer from occupational exposure to diesel exhaust. Subanalyses by study design (case-control and cohort studies) and by control for smoking produced results that did not differ from those of the overall pooled analysis. Cohort studies using internal comparisons showed higher relative risks than those using external comparisons. (See Figure 7-1.)

Bhatia and colleagues conclude that the analysis shows a small but consistent increase in the risk for lung cancer among workers with exposure to diesel exhaust. The authors evaluate the dependence of the relative risk estimate on the presence of control for smoking among studies, and provide a table that allows assessment of whether the quality of the data contributing to control for smoking is related to the relative risk estimates (albeit in a limited number of studies). Bhatia et al. assert that residual confounding is not affecting the summary estimates or conclusions for the following reasons: (1) the pooled relative risks for studies adjusted for smoking were the same as those for studies not adjusting for smoking; (2) in those studies giving risk estimates adjusted for smoking and risk estimates not adjusted for smoking, there was only a small reduction in the pooled relative risk from diesel exhaust exposure; and (3) in studies with internal comparison populations, in which confounding is less likely, the pooled relative risk estimate was 1.43.

The validity of this assessment depends on the adequacy of control for smoking in the individual studies. If inadequate adjustment for smoking is employed and residual confounding by cigarette smoking pertains in the result of the individual studies, then the comparisons and contrasts of the pooled estimates they cite as reasons for dismissing the effect of residual confounding by smoking will remain contaminated by residual confounding in the individual studies. In fact, Bhatia et al. erroneously identify the treatment of the smoking data in the main analysis for the 1987 report by Garshick et al. as a continuous variable representing pack-years of smoking, whereas the analysis actually dichotomized the pack-years data into two crude dose categories (above and below the 50 pack-years level). This clearly reduced the quality of the adjustment for smoking, which already suffered from the fact that information on cumulative cigarette consumption was missing for more than 20% of the lung cancer cases. In this instance, the consistency between the adjusted and unadjusted estimates of the relative risk for diesel exhaust exposure may be attributable to failure of adjustment rather than lack of confounding by

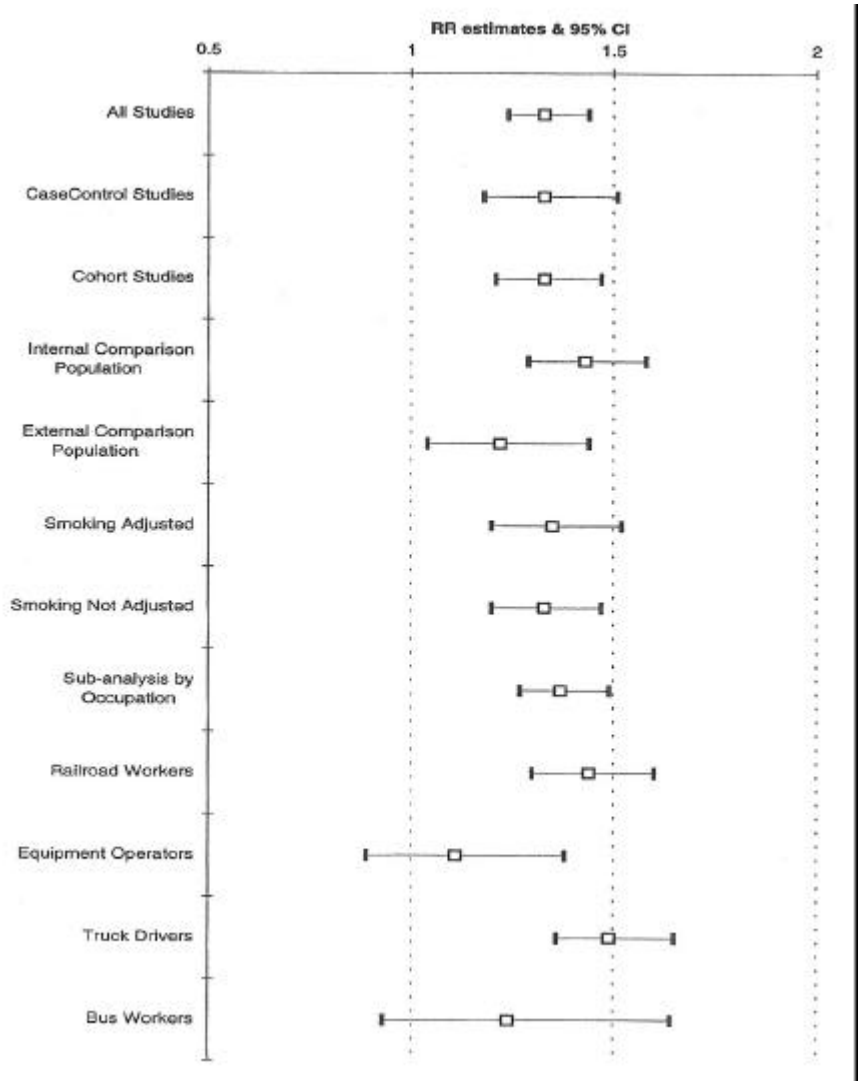


Figure 7-1. Pooled relative risk estimates and heterogeneity-adjusted 95% confidence intervals for all studies and subgroups of studies included in the meta-analysis.
Source: Bhatia et al., 1998.

cigarette smoking; and pooled estimates of association of diesel exhaust with lung cancer derived in the meta-analysis would remain confounded. A similar problem exists for the Bhatia et al. representation of the control for confounding in the study by Boffetta and Stellman (1988). Such mischaracterizations may indicate an overstatement by Bhatia et al. that the association of DE and lung cancer is insensitive to adjustment.

An evaluation of the potential for publication bias is presented that provides reassurance that the magnitude of published effects is not a function of the precision or study power; however, this assessment cannot rule out the possibility for publication bias.

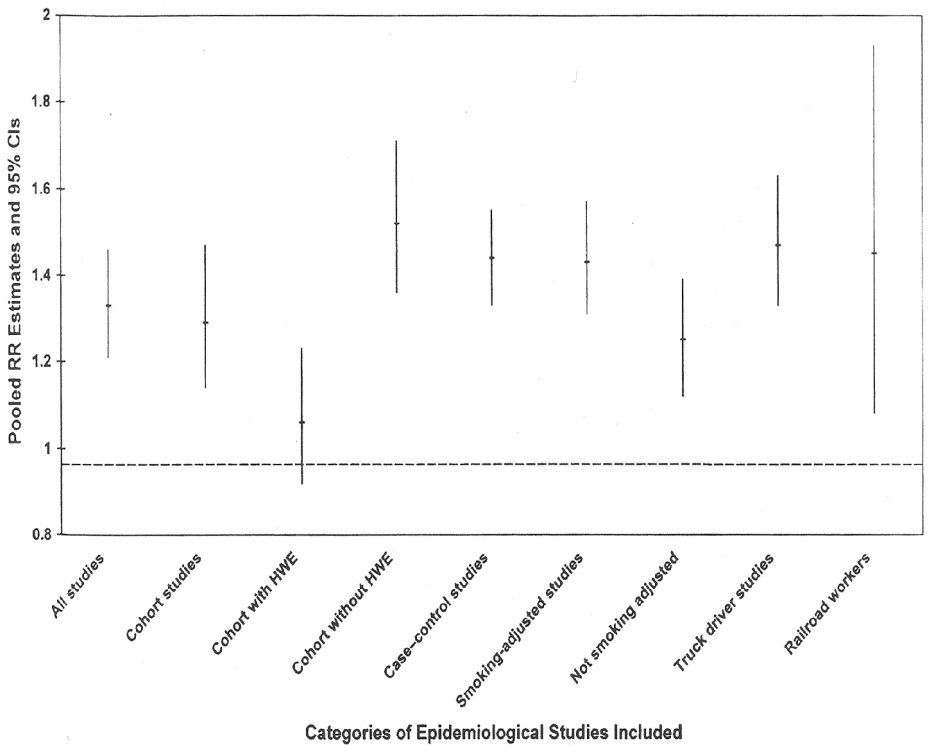
7.2.4.3. *Lipsett and Campleman (1999): Occupational Exposure to Diesel Exhaust and Lung Cancer: A Meta-Analysis*

Lipsett and Campleman (1999) conducted electronic searches to identify epidemiologic studies published between 1975 and 1995 of the relationship of occupational exposure to diesel exhaust and lung cancer. Studies were selected based on the following criteria. (1) Estimates of relative risks and their standard errors must be reported or derivable from the information presented. (2) Studies must have allowed for a latency period of 10 or more years for development of lung cancer after onset of exposure. (3) No obvious bias resulted from incomplete case ascertainment in followup studies. (4) Studies must be independent: that is, a single representative study selected from any set of multiple analyses of data from the same population. Studies focusing on occupations involving mining were excluded because of potential confounding by radon, arsenic, and silica, as well as possible interactions between cigarette smoking and exposure to these substances in lung cancer induction.

Thirty of the 47 studies initially identified as relevant met the specified inclusion criteria. Several risk estimates were extracted from six studies reporting results from multiple mutually exclusive diesel-related occupational subgroups. If a study reported effects associated with several levels or durations of exposure, the effect reported for the highest level or longest duration of exposure was used. If estimates for several occupational subsets were reported, the most diesel-specific occupation or exposure was selected. Adjusted risk estimates were used when available.

Thirty-nine independent estimates of relative risk and standard errors were extracted. Pooled estimates of relative risk were calculated using a random-effects model. Among study populations most likely to have had substantial exposure to diesel exhaust, the pooled smoking adjusted relative risk was 1.47 (95% CI = 1.29, 1.67). (See Figure 7-2.)

The between-study variance of the relative risks indicated the presence of significant heterogeneity in the individual estimates. The authors evaluated the potential sources of



Note. CI = confidence interval; HWE = healthy worker effect.

Figure 7-2. Pooled estimates of relative risk of lung cancer in epidemiological studies involving occupational exposure to diesel exhaust (random-effects models).

Source: Lipsett and Campleman, 1999.

heterogeneity by subset analysis and linear metaregressions. Major sources of heterogeneity included control for confounding by smoking, selection bias (a healthy worker effect), and exposure patterns characteristic of different occupational categories. A modestly higher, pooled relative risk was derived for the subset of case-control studies, which, unlike the cohort studies, showed little evidence of heterogeneity.

An evaluation of the potential for publication bias is presented that provides reassurance that the magnitude of published effects is not a function of the precision or study power; however, this assessment cannot rule out the possibility of publication bias.

Although a relatively technical approach was used in deriving summary estimates of relative risk and the evaluation of possible sources of variation in the relative risks in this meta-analysis, this approach should not be confused with rigorous evaluation of the potential weaknesses among the studies included in the analysis. The heterogeneity attributable to statistical adjustment for smoking was evaluated based on a dichotomous assessment of whether control for smoking could be identified in the studies considered. This does not reflect the adequacy of the adjustment for smoking employed in the individual studies considered. The potential for residual confounding by inadequate adjustment for the influence of smoking remains in the summary estimate of the relative risk.

7.2.5. Case-Control Studies of Bladder Cancer

7.2.5.1. *Howe et al. (1980): Tobacco Use, Occupation, Coffee, Various Nutrients, and Bladder Cancer*

This is a Canadian population-based case-control study conducted in the provinces of British Columbia, Newfoundland, and Nova Scotia. These areas were selected because they had cancer registries and were believed not to have concentrations of high-risk industries. All patients with newly diagnosed bladder cancer occurring in the three provinces between April 1974 and June 1976 were identified, and 77% of them were interviewed at home. A total of 480 male and 152 female case-control pairs were available for analysis. For each case, one neighborhood control, matched by age (±5 years) and sex, was also interviewed at home to obtain data on smoking, occupation, dietary sources of nitrites and nitrates that convert to nitrosamines (nonpublic water supply and preserved meat products), and beverage consumption, including a detailed history of coffee consumption. A detailed smoking history was obtained. The occupational history included a chronological account of all jobs and the number of years and months during which the respondent had worked in each job, experience in industries that were suspected a priori to increase the risk of bladder cancer, and exposure to any jobs that involved

exposure to dust and fumes at the workplace. Relative risk estimates were computed using the linear logistic model applied to individually matched case-control pairs.

A baseline comparison of cases and controls showed that, whereas male patients were similar to controls on income and education, there was an excess of female cases with low family incomes and low levels of educational attainment. For both sexes, the mean ages for cases and controls did not differ, and the times required for the interview were similar. An analysis by the a priori suspect industries showed elevated risks for a number of industries for males. These included the chemical (RR = 7.5, 95% CI = 1.7, 67.6), rubber (RR = 5.0, 95% CI = 0.6, 236.5), petroleum (RR = 5.3, 95% CI = 1.5, 28.6), medicine (RR = 2.6, 95% CI = 0.9, 9.3), and spray painting (RR = 1.8, 95% CI = 0.7, 4.6) industries. The excess risks were statistically significant only for the petroleum and chemical industries. The estimates did not change when the analysis was done separately for subjects who reported only one exposure and for those who reported exposure to more than one suspect industry. The estimates also remained unchanged after controlling for smoking. Too few females reported working in the a priori suspect industries to make any meaningful contribution to the analysis. Among males, statistically nonsignificant excess risks were observed for tanning, electric cable, photographic, commercial paint, tailoring, medicine, food processing, and agricultural industries. The analysis by exposure to dust and fumes in occupations other than those in the a priori suspect list detected the relative risks for diesel and traffic fumes (RR = 2.8, 95% CI = 0.8, 11.8). Statistically significant excess risks were observed for railroad workers (RR = 9.0, 95% CI = 1.2, 394.5) and welders (RR = 2.8, 95% CI = 1.1, 8.8). For occupations other than those on the a priori list for males and females, statistically significant excesses were detected for metal machinists (RR = 2.7, 95% CI = 1.1, 7.6), metal recorders (RR = 2.6, 95% CI = 1.0, 7.3), and nursery men (RR = 5.5, 95% CI = 1.2, 51.1). Statistically nonsignificant excesses were also detected for exposure to two chemicals: benzidine and its salts, RR = 1.3, and *bis*-chloromethyl ether, RR = 5.0. A detailed analysis was done for cigarette smoking, which demonstrated statistically significant increasing bladder cancer risk with increasing duration of smoking, total lifetime consumption of packs of cigarettes, and average frequency of cigarettes per day. In males the highest significant risk was observed for latency of less than 35 years; after that time the risk reduced slightly with increasing latency. In females the highest significant risk was for more than 35 years of latency. Risks were elevated for males consuming all types of coffee and for females consuming instant coffee. Hair dye usage in females and phenacetin usage in males and females carried no risk. Significant risks for use of artificial sweeteners and use of nonpublic water supplies (nitrates and nitrites) were found among males only.

This study was mainly designed to evaluate the various risk factors for bladder cancer such as smoking, coffee consumption, nitrates and nitrites in diet, and so on. The major limitation of this study, as the authors noted, was that the three selected provinces did not have high concentrations of industries suspected to be linked to bladder cancer. An excess risk was, however, detected for railroad workers and for those in the "exposed to diesel and traffic fumes category." Risks for those exposed to "diesel fumes only" were not available, nor do we know the exact job title of the railroad workers and the type of engines they were operating. The authors also did not detail the method by which they coded the information given by respondents in response to questions on exposure to dust and fumes into the various categories they used in the analysis. These analyses were done for subjects who reported having "ever been exposed" versus "never been exposed" to these fumes, and although detailed chronological work histories were obtained, no attempt was made to develop a lifetime cumulative exposure index to diesel fumes. In multiple logistic regression models, the authors used the a priori high-risk occupations; hence, nothing can be concluded about exposure to diesel exhaust for occupations that were not part of that list. The authors provided no explanation on possible selection bias, as only 77% of the eligible population was included in the study.

7.2.5.2. *Wynder et al. (1985): A Case-Control Study of Diesel Exhaust Exposure and Bladder Cancer*

A case-control study of diesel exhaust exposure and bladder cancer risk was conducted by Wynder et al. (1985). Cases and controls were obtained from 18 hospitals located in six U.S. cities between January 1981 and May 1983. Cases were individuals with histologically confirmed primary cancer of the bladder, diagnosed within 12 months before the interview. Controls were individuals with non-tobacco-related diseases who were matched to the cases by age (within 8 years), race, year of interview, and hospital of admission. Women were excluded from the study because the focus was on male-dominated occupations. A structured questionnaire was administered in the hospital to cases and controls to elicit information on usual occupation, smoking history, alcohol and coffee consumption, as well as other demographic factors.

Two methods were used to define occupational exposure to diesel exhaust. First, occupational titles defined by the industrial hygiene standards as probable high exposure were classified as exposed or not exposed to diesel exhaust. The probable high-exposure category consisted of bus and truck drivers, heavy equipment operators and repairmen, railroad workers, and warehousemen. In the second method, guidelines set by NIOSH were used to classify occupations based on exposure to diesel exhaust. In this method, the estimated proportion of

exposed workers was computed for each occupational category by using the NIOSH estimates of the exposed population as the numerator and the estimates of individuals employed in each occupational category from the 1970 census as the denominator. Occupations estimated to have at least 20% of their employees exposed to diesel exhaust were defined as "high exposure," those with 10% to 19% of their employees exposed as "moderate exposure," and those with less than 10% of their employees exposed as "low exposure." The odds ratio was used as a measure of association to assess the relationship between bladder cancer and diesel exhaust exposure. The overall participation among those eligible and available for interview was 75% and 72% in cases and controls, respectively.

A total of 194 bladder cancer cases and 582 controls were examined, and the two groups were found to be comparable by age and education. Except for railroad workers, who had relative odds of 2.0 based on two cases and three controls (95% CI = 0.34, 11.61), the relative odds were less than 1 for other diesel exhaust exposure occupations such as bus and truck drivers, warehousemen, material handlers, and heavy equipment workers. When the risk was examined using the NIOSH criteria for high, moderate, and low exposure, relative odds were 1.68 and 0.16 for high and moderate, respectively, with low as the referent group; neither was statistically significant. Cases and controls were compared by smoking status. Cases were more likely to be current cigarette smokers than were controls. Current smokers of 1 to 20 cigarettes/day had relative odds of 3.64 (95% CI = 2.04, 6.49), current smokers of 21+ cigarettes/day had relative odds of 3.51 (95% CI = 2.00, 6.19), while ex-smokers had relative odds of 1.72 (95% CI = 1.01, 2.92). After controlling for smoking, there was no significant increase in the risk of bladder cancer for occupations with diesel exhaust exposure compared with occupations without diesel exhaust exposure. A synergistic effect between the two also was not detected.

This study has two major methodologic limitations, both pertaining to exposure classification. First, the use of "usual" occupation may have led to misclassification of those individuals who had held a previous job with diesel exhaust exposure that was not their usual occupation; this may have resulted in reduced power to detect weak associations. Second, since there was no information on amount or duration of diesel exhaust exposure, no analysis of dose-response relationships could be done. Also, no information was available on other confounding risk factors of bladder cancer such as urinary retention, amphetamine abuse, and smoking within the confined space of a truck cab, all of which are lifestyle factors specific to the truck-driving occupation.

7.2.5.3. *Hoar and Hoover (1985): Truck Driving and Bladder Cancer Mortality in Rural New England*

This study investigated the relationship between the occupation of truck driving and bladder cancer mortality in a case-control study in New Hampshire and Vermont. Cases included all white residents of New Hampshire and Vermont who died from bladder cancer (eighth revision of the ICD) between 1975 and 1979. Death certificates were provided by the vital records and health statistics office of the two States, and the next of kin were traced and interviewed in person. Two types of controls were selected for each case. One control was randomly selected from all other deaths, excluding suicides, and matched on State, sex, race, age (±2 years), and year of death. The second control was selected with the additional matching criterion of county of residence. Completed interviews were obtained from 325 (out of 410) next of kin for cases and 673 (out of 923) for controls. Information on demographic characteristics, lifetime occupational and residential histories, tobacco use, diet, and medical history was obtained on each subject. The odds ratio was calculated to ascertain a measure of association between truck driving and bladder cancer. Because separate analyses of the two control series gave similar results, the two control series were combined. Also, because matched analyses yielded results similar to those provided by the unmatched analyses, results of the unmatched analyses were presented.

Sixteen percent (35) of the cases and 12% (53) of the controls had been employed as truck drivers, yielding an odds ratio of 1.5 (95% CI = 0.9, 2.6) after adjustment for county of residence and age at death. For New Hampshire, the odds ratio was 1.3 (95% CI = 0.7, 2.3), and for Vermont, the odds ratio was 1.7 (95% CI = 0.8, 3.4). For a large number of subjects, the next of kin were unable to give the durations of truck driving, and there was an inconsistent positive association with years of truck driving. Crude relative odds were not altered after adjustment for coffee drinking, cigarette smoking, and education as a surrogate for social class. Little variation in risks was seen when the data were analyzed by the industry in which the men had driven trucks. No relationship was seen between age at which employment as a truck driver started and occurrence of bladder cancer. Analysis by duration of employment as a truck driver and bladder cancer showed a positive trend of increasing relative odds with increasing duration of employment. The trend test was statistically significant (p=0.006). The odds ratio was statistically significant for the 5 to 9 years of employment category only (OR = 2.9, 95% CI = 1.2, 6.7). Similarly, analysis by calendar year first employed showed a statistically significant odds ratio for 1930 to 1949 (OR = 2.6, 95% CI = 1.3, 5.1), whereas relative odds were not significant if subjects were employed prior to 1929 or after 1950.

The effects of reported diesel exhaust exposure from fuel or engines in truck driving or other occupations were then analyzed. An odds ratio of 1.8 (95% CI = 0.5, 7.0) was derived for those who were exposed to diesel exhaust during their truck-driving jobs as compared to an odds ratio of 1.5 (95% CI = 0.8, 2.7) for those not reporting diesel exhaust exposure. Analysis by duration of exposure (0, 1 to 19 years, 20 to 29 years, 30 to 39 years, and 40+ years) to diesel fuel or engines in other occupations, which were "self-reported" by participants, showed a statistically significant positive trend (p=0.024) for bladder cancer, although none of the individual odds ratios in these duration categories were statistically significant.

This study investigated an association between truck driving and bladder cancer in an attempt to understand the reasons for the high rates of bladder cancer in rural areas of New Hampshire and Vermont. Although an elevated odds ratio for bladder cancer (not statistically significant) was observed for reported truck-driving occupations, there was insufficient evidence to conclude that the excess risk of bladder cancer was due to exposure to diesel emissions. This is because the excess bladder cancer risk was observed for all truck drivers irrespective of their exposure to diesel emissions. Also, no information was provided on the confounding effects of other aspects of the road environment such as urinary retention, amphetamine abuse, and concentrated cigarette smoke within the truck cab. Other limitations of this study include the use of next of kin for occupational histories, who may either under- or overreport occupations, and the use of death certificates with their inherent problems of misclassification.

7.2.5.4. *Steenland et al. (1987): A Case-Control Study of Bladder Cancer Using City Directories as a Source of Occupational Data*

The primary objective of the study was to test the usefulness of city directories as a source of occupational data in epidemiologic studies of illness and occupational exposure. Commercial city directories provide data on occupations and employers and are compiled from a door-to-door yearly census of all residents 18 years old and older. The directories are available in most medium-size cities in the United States. A unique feature of city directory data is that they identify specific employers, and as the authors suggest, this information may be better than death certificates for rapid, inexpensive, record-based epidemiologic studies.

A case-control study was conducted of male bladder cancer deaths in Hamilton County (including Cincinnati), OH. This county was selected because it is in an industrialized area with high bladder cancer rates and also because city directories cover approximately 85% of the people living in the county. A computerized list of all male bladder cancer deaths (n = 731) and all other male deaths (n = 95,057), with the exclusion of deaths from urinary tract tumors and pneumonia, that occurred between 1960 and 1982 was obtained from the Ohio Department of Vital Statistics.

Death certificates had been coded by a nosologist according to the ICD code in use at the time of death. A pool of six controls was created for each case matched on sex, residence in Hamilton County at time of death, year of death, age at death (±5 years), and race.

Two types of analysis were performed, one based on city directory data and the other based on death certificate data. In the former, cases and controls were restricted to individuals who had at least one yearly directory listing with some occupational data. The first two controls from the pool of six who met the requirements were selected. This analysis involved 648 cases (627 cases had 2 controls and 21 cases had only 1 control) and 1,275 controls.

The death certificate analysis involved all 731 cancer deaths, with two controls per case. In most cases, the same two controls were used in this analysis too. The usual lifetime occupation and industry on the death certificate was abstracted from them. Data on occupation and industry were coded with a three-digit U.S. census code using the method adopted by the U.S. Bureau of the Census. Five of the occupational data were recorded for occupation and industry by a second coder, with a high degree of reproducibility. Odds ratios were calculated for bladder cancer using a Mantel-Haenszel procedure.

The city directory data identified four employers significantly associated with bladder cancer deaths; only one of them was identified by the death certificate data, which provided only lifetime type of industry rather than the name of a specific employer. The industries represented by the four employers were a chemical plant, printing company, valve company, and machinery plant. The city directory data analysis demonstrated significant positive associations for quite a few occupations. The occupations that had at least 10 cases or more were engineers (OR = 3.00, p=0.01), carpenters (OR = 2.36, p<0.01), tailors (OR = 2.56, p<0.01), and furnace operators (OR = 2.5, p=0.03). Relative odds were increased significantly with increased duration of employment ($\geq$20 years) for truck drivers (OR = 12, p=0.01) and furnace operators (based on four cases and no controls, p=0.05). For occupations in which subjects had ever been employed, a significant increase in the relative odds with increased duration of employment was observed for the railroad industry ($\geq$20 years of employment, OR = 2.21, p<0.05). Both truck driving and railroad industry occupations involve diesel emission exposures.

The analysis of death certificate data yielded associations in the same direction for most of the occupations. A check of the validity of city directory data indicated that 77% of the listings agreed with the Social Security earnings report for the employer in any given year. A comparison of city directory and death certificate information on occupations indicated a match for occupation between at least one city directory listing and occupation on death certificates for 68.3% of the study subjects.

This study demonstrated that city directories are a relatively inexpensive and accessible source of occupational data for epidemiologic studies. Limitations of this study include the problem in tracing women because of the change from maiden to married name and the availability of data for only the year of residence in the city. They are superior to death certificates in being able to identify high-risk employers in specific geographic sites. Although death certificate data reflect usual lifetime occupation, city directories yield data on short-term jobs, some of which may involve critical exposure. Thus, a combination of the two approaches may be most productive in record-based hypothesis-generating studies. The occupational data were missing for 15%, whereas employer data were missing for 36% in the city directory. In the context of the mentioned pros and cons of using city directories, this study found an excess risk for bladder cancer among two occupations with potential diesel exposure: truck drivers and railroad workers. Two sources of bias that may have influenced these findings are selection bias arising from the use of deaths instead of incident cases, because survival for bladder cancer is high, and the absence of data on confounding factors such as smoking, beverage consumption, and medication use.

7.2.5.5. *Iscovich et al. (1987): Tobacco Smoking, Occupational Exposure, and Bladder Cancer in Argentina*

This is a hospital-based case-control study of bladder cancer conducted in La Plata, Argentina, to estimate the risk of bladder cancer associated with different types of tobacco, beverages, and occupational exposures. Bladder cancer is one of the most common cancers among males in the La Plata area.

Cases were selected from patients with a histologically confirmed diagnosis of bladder cancer (transitional, squamous-cell, or nonspecific cell type) admitted to the 10 general hospitals in the greater La Plata area (population in 1980 = 580,000) between March 1983 and December 1985. Cases with true bladder papilloma and individuals who were residents of greater La Plata for less than 5 years were excluded. Of the 120 cases eligible to participate, 1 died prior to the interview, 2 refused to participate, and the remaining 117 cases, representing 60% of the incident cases registered in the registry, were interviewed. Two control groups (117 neighborhood and 117 hospital controls) were matched by sex and age (±5 years). Of the 117 cases, 99 were males and 18 were females. Hospital controls, selected from the same hospital as the cases, were hospitalized for the first time within 3 months of diagnosis of the illness of the cases. Twelve percent of the hospital controls had illnesses known to be associated with tobacco smoking. Neighborhood controls were sampled from among persons living in the same block. The interviewer proceeded north in the block where the case resided and selected the first control who

met the matching criteria. Seven hospital controls could not be interviewed because of their poor physical health and were replaced. Three neighborhood controls refused to participate and were replaced. Cases and hospital controls were interviewed at the hospital and the neighborhood controls at their homes to collect data on demographic, socioeconomic, and medical variables, detailed smoking habits, and alcoholic and other beverages consumed.

The interviews were done by trained interviewers, two physicians and a social worker. The cases and hospital controls were interviewed in the hospital by the physicians; hence, the interviews could not be conducted "blind." A detailed occupational history was obtained for the three occupations of longest duration and the most recent one. For each job title, the activity of the plant and type of production were also ascertained. Job titles were coded according to the International Labor Union (ILO) 1970 classification. Plant activity and type of production were coded according to the United Nations 1975 classification categories. Information was also collected on exposure to 33 chemical and physical agents, which included confirmed or suspected bladder carcinogens. A detailed history of smoking habits was also obtained, and individuals were categorized as current smokers if they were smoking at present or if they had stopped smoking less than 2 years previously. Ex-smokers were those who ceased smoking at least for 2 years or more than 2 years previously. For each subject a cumulative lifelong consumption of cigarettes by type was estimated, and an average consumption of cigarettes/day was computed.

Relative risks were computed for occupational factors using the unconditional logistic regression method, adjusting for age and tobacco smoking. These risks were derived for those who were ever employed in that occupation versus those who were never employed in that occupation. Socioeconomic status of cases and neighborhood controls was similar, but there were fewer professionals and more manual workers among hospital controls compared with cases. Occupational variables included job title and type of activity of the plant. Significant excess risks were observed for truck and railroad drivers (RR = 4.31, $p<0.002$) and oil refinery workers (RR = 6.22, $p<0.02$). The risk for truck and railroad drivers was reduced after adjusting for smoking, whereas that for oil refinery workers increased after adjusting for smoking (no RRs were presented). The adjusted relative risks were not reported. A positive but nonsignificant association was observed for printers (RR = 2.6, $p<0.77$).

This study identified smoking and coffee drinking as the major risk factors for bladder cancer in this area. The overall age-adjusted relative risk in males for current smokers relative to nonsmokers was 7.2 (95% CI = 3.0, 20.1), with dose-response relationships observed for the average daily amount as well as for duration of smoking. A strong dose-response relationship was also observed for coffee drinking in males, with a relative risk of 12 (95% CI = 4.3, 33.31) for those drinking more than three cups of coffee per day after adjusting for the effect of smoking.

No association was found with use of saccharin in males. No results were presented for females for these risk factors.

This case-control study was conducted primarily to determine the reasons for the high rates of bladder cancer in the La Plata region of Argentina. Only 60% of the cases registered in the cancer registry were interviewed, and no information was provided for the remaining 40% eligible nonrespondents to determine if the study sample was selectively biased in any way. The sample size of 117 was small, and the analysis of males reduced it to 99. Although the use of two different types of control groups is a strength of this study, none of the interviews were done blind, and it appears that the hospital interviews were done by the physicians and the neighborhood interviews were done by the social worker. Job titles were used as surrogates of exposure, but the authors state that although they attempted to analyze by an exposure index derived from a job exposure matrix (details not provided), they found no difference in exposure between cases and controls. This explanation is ambiguous. The authors also grouped truck and railroad drivers together for reasons not mentioned and did not present separate risk estimates. A table showing the distribution of cases and controls for selected activities or professions did not indicate if the data pertain to both sexes or males only, and the text did not clarify that either. The reported significant excess risks for truck and railroad drivers were reduced after adjusting for smoking, but it was not known if the statistical significance persisted. No analysis was provided for the data collected in the interviews on exposures to the 33 chemical and physical agents, and it was not known if the truck and railroad drivers were operating diesel engines. Although rare in the La Plata area, the occupations known to be associated with bladder cancer (dye, rubber, leather, and textile workers) are acknowledged by the authors.

7.2.5.6. *Iyer et al. (1990): Diesel Exhaust Exposure and Bladder Cancer Risk*

This study is a hospital-based case-control study of bladder cancer and potential exposure to diesel exhaust using data from a large ongoing case-control study of tobacco-related neoplasms conducted by the American Health Foundation. An earlier study by Wynder et al. (1985) looked at the relationship between occupational exposure to diesel exhaust and the risk of bladder cancer. For this study, the objective was to evaluate the relationship between the different measures of exposure to diesel exhaust, occupational and self-reported, and the risk of bladder cancer. Cases comprised 136 patients with histologically confirmed primary cancer of the urinary bladder interviewed at 18 hospitals in six U.S. cities. Two controls were selected per case, matched for sex, age (within 2 years), race, hospital, and year of interview. A total of 160 controls had non-tobacco-related malignancies distributed as follows: stomach cancer (6%), colorectal cancer (20%), prostate cancer (6%), and leukemia or lymphoma (8%). Among the 112 controls with

nonmalignant diseases, 3% had benign neoplasms, 6% had hyperplasia of the prostate, and 6% had dorsopathies. Distribution of the other nonmalignant illnesses was not provided. Occupational history included information on usual occupation and up to five other jobs. Exposure to diesel exhaust in hobby activities also was collected. For the purpose of this analysis, occupations were aggregated a priori into three categories: low probability of exposure (reference group), possible exposure, and probable exposure. Analyses were also done for self-reported exposure to diesel exhaust. Risk estimates were obtained by unconditional logistic regression using PROC LOGIST of SAS. Cases and controls were first compared by age, race, education, and smoking habit. Cases were found to be less educated than controls ($p<0.05$). Crude odds ratios for diesel exhaust exposure, based on occupational or self-reported exposure, were not significantly elevated after controlling for smoking and educational status (OR = 1.2, 95% CI = 0.8, 2.0). When individual occupations were analyzed separately, truck drivers showed no excess risk (OR = 0.48, 95% CI = 0.15, 1.56).

The authors concluded that their study does not support the hypothesis of an association between exposure to diesel exhaust and bladder cancer. Several significant limitations of exposure assessment and analysis are evident in this study. In the introduction, the authors stated that they refined the definition of exposure to diesel exhaust by obtaining a lifetime occupational history, but in the methods section they stated that they restricted analysis to usual occupational history and five other jobs, which was not that different from their earlier study (Wynder et al., 1985). The terms, low probability of exposure, possible exposure, and probable exposure, also were not clearly defined. Information on duration of employment or exposure was not presented, and no attempts were made to validate occupational history. No information was available on calendar years of employment in the truck-driving industry or the locomotive occupations. Because diesel trucks and locomotives were introduced in the mid-1950s and the dieselization was completed by 1960, it would be important to use 1960 as a cutoff date and to restrict analysis to subjects who worked in these industries after that date. No information on nonrespondent cases and controls was provided. The authors indicated in the methods section that cases were individually matched to controls, but they performed an unmatched analysis to calculate the odds ratios and did not address why they did not preserve the matching in the analysis, especially because such an analysis could bias the risk estimates to unity.

7.2.5.7. *Steineck et al. (1990): Increased Risk of Urothelial Cancer in Stockholm From 1985 to 1987, After Exposure to Benzene and Exhausts*

This study was conducted to investigate the association between benzene, diesel, and petrol exhausts as well as some other industry-related chemicals and the risk of urothelial cancer. Designed as a population-based case-control study, it was conducted among all men born

between 1911 and 1945 and living in the County of Stockholm for all or part of the observation period (September 15, 1985, to November 30, 1987). All incident cases of urothelial cancer and squamous-cell carcinoma of the lower urinary tract were contacted for inclusion in the study. Controls were selected by stratified random sampling during the observation period from a computerized register for the population of Stockholm. A postal questionnaire was sent to study subjects at their homes to collect information on occupational history. The questions on occupation included exposure to certain specified occupations/industries/chemicals and lists of all jobs held and duration in each job. An industrial hygienist, unaware of case-control status, classified subjects as being exposed or unexposed to 38 agents and groups of substances, including 17 exposure categories with aromatic amines. Using all the exposure information, the exposure period was defined and the annual dose was rated as low, moderate, or high based on the accumulated dose (exposure duration multiplied by intensity of exposure) during the course of 1 average year for the defined exposure period. Swedish and international data were used to classify subjects as exposed, based on air concentrations in the work environment that were higher than for the general public, or skin contact with liquids of low volatility. To allow for latency, the authors ignored exposures after 1981. Data were gathered from 256 cases and 287 controls. Controls were selected by stratified random sampling four times from the computerized register during the observation period of the population of the County of Stockholm. These subjects comprised 80% of eligible cases and 79% of eligible controls. Nine of the cases and 16% of the controls refused to participate in the study.

The distribution of urothelial cancers was as follows: 5 tumors in the renal pelvis, 243 in the urinary bladder, 5 in the ureter, none in the urethra, and 3 at multiple sites. Two cases who were exposed to a high annual dose of aromatic amines were omitted from all further analysis to eliminate their confounding effects. Crude relative risks were calculated for men classified as exposed or not exposed to several substances. Twenty-five cases and 19 controls reported having been exposed to diesel exhaust, yielding an odds ratio of 1.7 (95% CI = 0.9, 3.3). The corresponding relative odds for petrol exhausts, based on 24 cases and 24 controls, were 1.0 (95% CI = 0.5, 1.9). Odds ratios were then calculated for low, moderate, and high levels of the annual dose adjusted for smoking and year of birth. For diesel exhausts, the odds ratio was 1.3 (95% CI = 0.6, 3.1) for low levels, 2.2 (95% CI = 0.7, 6.6) for moderate levels, and 2.9 (95% CI = 0.3, 30.0) for high levels, indicating a dose response. The corresponding odds ratios for petrol exhausts were 0.6 (95% CI = 0.3, 1.3), 1.4 (95% CI = 0.5, 3.7), and 3.9 (95% CI = 0.4, 35.5).

Restricting the analysis to only moderate or high annual doses of exposure adjusted for year of birth and smoking showed a sevenfold increased risk for subjects exposed to both diesel and petrol exhausts (OR = 7.1, 95% CI = 0.9, 58.8). For exposure to diesel (OR = 1.1) and

petrol (OR = 1.0) exhausts alone, no excess risk was detected in this analysis. Odds ratios were calculated for low, moderate, and high exposure to benzene, with rates of 1.7 (95% CI = 0.6, 5.1) for low annual doses, 1.1 (95% CI = 0.3, 4.5) for moderate annual doses, and 3.0 (95% CI = 1.0, 8.7) for high annual doses.

The authors discuss misclassification and confounding as sources of bias in this study. To examine misclassification they compared hygienist-assessed exposure and self-reported exposure for printing ink and found a higher relative risk and fewer exposed subjects for hygienist-assessed exposure, indicating that specificity was a problem for self-reported exposure. It is not known to what extent this may have affected the risk estimates for diesel exhausts since data on self-reported exposure to diesel are not presented. They also mention the possibility of exposure misclassification from using an average annual dose in which a person exposed to an agent at a high level for a few working days and a person exposed to a low level for many days are both rated as exposed to low annual doses. Although statistically nonsignificant elevated odds ratios of 1.3, 2.3, and 2.9 were derived for low, moderate, and high levels of diesel exposure, the authors state that some of their subjects may have later worked in jobs with benzene exposure, and because an elevated risk was detected for benzene exposure, this confounding effect may explain some of the excess risk. An almost statistically significant interaction was observed for exposure to combined diesel and petrol exhausts (OR = 7.1, 95% CI = 0.9, 58.8), which changed to 5.1 (95% CI = 0.6, 43.3) after adjustment for benzene exposure, again providing evidence for the confounding role of benzene exposure in explaining some of the observed results.

Table 7-3 summarizes the bladder cancer case-control studies.

7.2.6. Discussion and Summary

Certain extracts of diesel exhaust have been demonstrated as both mutagenic and carcinogenic in animals and in humans. Animal data suggest that diesel exhaust is a pulmonary carcinogen among rodents exposed by inhalation to high doses over long periods of time. Because large working populations are currently exposed to diesel exhaust and because nonoccupational ambient exposures currently are of concern as well, the possibility that exposure

Table 7-3. Epidemiologic studies of the health effects of exposure to diesel exhaust: case-control studies of bladder cancer

Authors	Population studied	Diesel exhaust exposure	Results	Limitations
Howe et al. (1980)	480 male case-control pairs 152 female case-control pairs Cases diagnosed between April 1974 and June 1976 in three Canadian provinces Matched on age and sex	Based on occupational history of jobs involving exposure to dust and fumes A priori suspect industries	SNS RR = 2.8 for diesel and traffic fumes SS RR = 9.00 for railroad workers	Exposure based on occupational history, which was not validated Diesel exhaust and traffic fumes were combined Only 77% of eligible population included in the study
Wynder et al. (1985)	194 histologically confirmed male cases between the ages of 20 and 80 years 582 matched controls (age, race, year of interview, and hospital of admission); diseases not related to tobacco use From 18 hospitals located in six U.S. cities between January 1981 and May 1983	Occupational titles were defined by Industrial Hygiene Standard into dichotomous "exposed" and "not exposed" Also defined by NIOSH standards into "high exposure," "moderate exposure," and "low exposure"	SNS ORs were 1.68 and 0.16 for high and moderate exposure, respectively, as compared to low exposure	Exposure based on usual occupation may have led to misclassification Dichotomous classification made dose-response analysis unattainable No data on other confounders such as smoking

Table 7-3. Epidemiologic studies of the health effects of exposure to diesel exhaust: case-control studies of bladder cancer (continued)

Hoar and Hoover (1985)	Population-based, case-control study 325 cases from the residents of New Hampshire and Vermont who died of bladder cancer between 1975 and 1979 A total of 673 controls were chosen from other deaths during the same time period Two matched controls (age, sex, race, state, year of death, and second one matched also on	Lifetime occupational history obtained from next of kin	SS OR = 2.9 for 5 to 9 years of employment as truck driver but not for ≥10 years of employment Positive trend (p=0.006) observed with increasing duration of employment as truck driver	Exposure defined as occupation of "truck driver" (i.e., it could have been diesel or gasoline or both) No histological confirmation of bladder cancer diagnosis No data on other confounders such as other exposures, smoking, etc.
Steenland et al. (1987)	648 male bladder cancer deaths from Hamilton County, OH 1,275 matched controls from other deaths (pool of six controls for each case, excluding urinary tract tumors and pneumonias matched on sex, age at death, year of death, race)	Occupation or industry listed in city directory and on death certificates	OR = 12 (p=0.01) for truck drivers with ≥20 years of employment OR = 2.21 ($p \leq 0.05$) for railroad workers with >20 years of employment	Exposure based on city directory or death certificate listing that was not validated Lack of controlling for confounders City directory usually has short-term job listing Missing data on 15% of occupations and 36% for employers in the directory

Table 7-3. Epidemiologic studies of the health effects of exposure to diesel exhaust: case-control studies of bladder cancer (continued)

Iscovich et al. (1987)	117 histologically confirmed bladder cancer cases (60% of all incident cases) 117 hospital controls and 117 neighborhood controls (matched on age and sex) Cases and hospital controls from 10 general hospitals in greater La Plata between March 1983 and December 1985	Past and present occupational data were collected by questionnaire An exposure index based on a job exposure matrix was generated	SS OR = 4.3 for truck and railway drivers SS RR = 6.2 for oil refinery workers	Exposure based on job held that was not validated 40% of eligible cases were nonrespondent Small sample size Interviewers were not "blind" to the status of an individual, and this could have biased the findings Truck and railroad drivers were grouped together Not adjusted for other confounding
Iyer et al. (1990)	136 histologically confirmed bladder cancer cases 272 controls, two each matched on sex, age, race, hospital, and year of interview (160 malignant, 112 nonmalignant) From 18 hospitals in six U.S. cities	Lifetime occupational history Self-reported diesel exhaust exposure Exposure aggregated a priori into: Low probability Possible Probable	No excess found	Exposure based on self-report, which was not validated Although lifetime occupational history was obtained, analysis was restricted to usual occupation A priori classification was ambiguous

Table 7-3. Epidemiologic studies of the health effects of exposure to diesel exhaust: case-control studies of bladder cancer (continued)

Steineck et al. (1990)	Population-based study from County of Stockholm Men born between 1911 and 1945 256 (243 bladder) urinary tract cancer incident cases (80% of eligibles) 287 controls (79% of eligibles) from population of Stockholm Observation period September 15, 1985, to November 30, 1987	Occupational history classified into exposed and nonexposed by industrial hygienist "blind" toward case or control status Using all exposure information, annual dose rated as "low", "moderate," and "high"	SNS OR = 1.3 for low, OR = 2.2 for moderate, and OR = 2.9 for high exposure were observed for diesel exposure SNS OR = 7.1 observed for diesel and gasoline exhaust combined exposure	Elaborate exposure history classification not used to advantage by simultaneous adjustment Misclassification in exposure may have occurred Small sample size of only 25 cases and 19 controls were exposed to diesel exhaust Confounding by other exposures not accounted for, except benzene

Abbreviations: OR = odds ratio; RR = relative risks; SNS = statistically nonsignificant; SS = statistically significant.

to this complex mixture may be carcinogenic to humans has become an important public health issue.

Because diesel emissions become diluted in the ambient air, it is difficult to study the health effects in the general population. Nonoccupational exposure to diesel exhaust is worldwide in urban areas. Thus, "unexposed" reference populations used in occupational cohort studies are likely to contain a substantial number of individuals who are nonoccupationally exposed to diesel exhaust. Furthermore, the "exposed" group in these studies is based on job titles, which in most instances are not verified or correlated with environmental hygiene measurement. The issue of health effect measurement is further complicated by the fact that occupational cohorts tend to be healthy and have below-average mortality, usually referred to as the "healthy worker effect." Hence, the usual standard mortality ratios observed in cohort mortality studies are underestimations of real risk.

A major difficulty with the occupational studies considered here was measurement of actual diesel exhaust exposure. Because all the cohort mortality studies were retrospective, assessment of health effects from exposure to diesel exhaust was naturally indirect. In these occupational settings, no systematic quantitative records of ambient air were available. Most studies compared men in job categories with presumably some exposure to diesel exhaust with either standard populations (presumably no exposure to diesel exhaust) or men in other job categories from industries with little or no potential for diesel exhaust exposure. A few studies have included measurements of diesel fumes, but there is no standard method for the measurement. No attempt is made to correlate these exposures with the cancers observed in any of these studies, nor is it clear exactly which extract should have been measured to assess the occupational exposure to diesel exhaust. All studies have relied on the job categories or self-report of exposure to diesel exhaust. In the studies by Garshick et al. (1987, 1988), the diesel exhaust exposed job categories were verified on the basis of an industrial hygiene survey done by Woskie et al. (1988a,b). The investigators found that in most cases the job titles were good surrogates for diesel exhaust exposure. Also, in the railroad industry, where only persons who had at least 10 years of work experience were included in the study, the workers tended not to change job categories over the years. Thus, a job known only at one point in time was a reasonable marker of past diesel exhaust exposure. Unfortunately, the exposure was only qualitatively verified. Quantitative use of this information would have been much more meaningful. Occupations involving potential exposure to diesel exhaust are miners, truck drivers, transportation workers, railroad workers, and heavy equipment operators.

With the exception of the study by Waxweiler et al. (1973), no known studies of miners have assessed whether diesel exhaust is associated with lung cancer. Currently, there are about 385 underground metal mines in the United States. Of these, 250 have been permanently

operating and 135 have been intermittently operating (Steenland, 1986). Approximately 20,000 miners are employed, but not all of them are currently working in the mines. Diesel engines were introduced in the metal mines in the early to mid-1960s. Although all these mines use diesel equipment, it is difficult to estimate how many of these miners were actually exposed to diesel fumes.

Diesel engines were introduced in coal mines at an even later date, and their use is still quite limited. In 1983, approximately 1,000 diesel units were in place in underground coal mines, up from about 200 units in 1977 (Daniel, 1984). The number of units per mine varies greatly; one mine may account for more than 100 units.

Even if it were possible to estimate how many miners (metal and coal) were exposed to diesel exhaust, it would be very difficult to separate out the confounding effects of other potential pulmonary carcinogens, such as radon decay products or heavy metals (e.g., arsenic, chromium). Furthermore, the relatively short latency period limits the usefulness of these cohorts of miners.

7.2.6.1. *The Cohort Mortality Studies*

The cohort studies mainly demonstrated an increase in lung cancer. Studies of bus company workers by Waller (1981), Rushton et al. (1983), and Edling et al. (1987) failed to demonstrate any statistically significant excess risk of lung cancer, but these studies have certain methodological problems, such as small sample sizes, short followup periods (just 6 years in the Rushton et al. study), lack of information on confounding variables, and lack of analysis by duration of exposure, duration of employment, or latency that preclude their use in determining the carcinogenicity of diesel exhaust. Although the Waller (1981) study had a 25-year followup period, the cohort was restricted to employees (ages 45 to 64) currently in service. Employees who left the job earlier, as well as those who were still employed after age 64 and who may have died from cancer, were excluded.

Wong et al. (1985) conducted a mortality study of heavy equipment operators that demonstrated a significantly increased risk of liver cancer in total and in various subcohorts. The same analysis also showed statistically significant deficits in cancers of the large intestine and rectum. Metastases from the cancers of the large intestine and rectum in the liver probably were misclassified as primary liver cancer, which led to an observed excess risk. This study did demonstrate a nonsignificant positive trend for cancer of the lung with length of membership and latency. Analysis of deceased retirees showed a significant excess of lung cancer. Individuals without work histories who started work prior to 1967, when records were not kept, may have been in the same jobs for the longest period of time. Workers without job histories included those who had the same job before and after 1967 and thus may have worked about 12 to 14 years

longer; these workers exhibited significant excess risks of lung cancer and stomach cancer. If this assumption about duration of jobs is correct, then these site-specific causes can be linked to diesel exhaust exposure. One of the methodological limitations of this study is that most of these men worked outdoors; thus, this cohort might have had relatively low exposure to diesel exhaust. The authors did not present any environmental measurement data either. Because of the absence of detailed work histories for 30% of the cohort and the availability of only partial work histories for the remaining 70%, jobs were classified and ranked according to presumed diesel exposure. Information is lacking regarding duration of employment in the job categories (used for surrogate of exposure) and other confounding factors (alcohol consumption, cigarette smoking, etc.). Thus, this study cannot be used to support a causal association or to refute the same between exposure to diesel exhaust and lung cancer.

A 2-year mortality analysis by Boffetta and Stellman (1988) of the American Cancer Society's prospective study, after controlling for age and smoking, demonstrated an excess risk of lung cancer in certain occupations with potential exposure to diesel exhaust. These excesses were statistically significant among miners (RR = 2.67, 95% CI = 1.63, 4.37) and heavy equipment operators (RR = 2.6, 95% CI = 1.12, 6.06). The elevated risks were nonsignificant in railroad workers (RR = 1.59) and truck drivers (RR = 1.24). A dose response was also observed for truck drivers. With the exception of miners, exposure to diesel exhaust occurred in the three other occupations showing an increase in the risk of lung cancer. Despite methodologic limitations, such as the lack of representiveness of the study population (composed of volunteers only, who were probably healthier than the general population), leading to an underestimation of the risk and the questionable reliability of exposure data based on self-administered questionnaires that were not validated, this study is suggestive of a causal association between exposure to diesel exhaust and excess risk of lung cancer.

Two mortality studies were conducted by Gustavsson et al. (1990) and Hansen (1993) among bus garage workers (Stockholm, Sweden) and truck drivers, respectively. An SMR of 122 was found among bus garage workers based on 17 cases. A nested case-control study was also conducted in this cohort. Detailed exposure matrices based on job tasks were assembled for both diesel exhaust and asbestos exposures. Statistically significant increasing lung cancer relative risks of 1.34, 1.81, and 2.43 were observed for diesel exhaust indices of 10 to 20, 20 to 30, and >30, respectively, using 0 to 10 as a comparison group. Adjustment for asbestos exposure did not change the results. The main strength of this study is the detailed exposure matrices; some of the limitations are lack of smoking histories and low power (small cohort).

Hansen (1993), on the other hand, found statistically significant SMR of 160 due to cancer of bronchus and lung. No dose response was observed, although the excesses were

observed in most of the age groups (30 to 39, 45 to 49, 50 to 54, 55 to 59, 60 to 64, and 65 to 74). There are quite a few methodologic limitations to this study. Exposure to diesel exhaust was assumed in truck drivers for diesel-powered trucks, but no validation of exposure was attempted. Smoking data were lacking, followup period was short, and no latency analysis was done. The findings of both these studies are consistent with the findings of other truck driver studies.

Two mortality studies of railroad workers were conducted, by Howe et al. (1983) in Canada and Garshick et al. (1988) in the United States. The Canadian study found relative risks of 1.2 (p<0.01) and 1.35 (p<0.001) among "possibly" and "probably" exposed groups, respectively. The trend test showed a highly significant dose-response relationship with exposure to diesel exhaust and the risk of lung cancer. The main limitation of the study was the inability to separate overlapping exposures of coal dust and diesel fumes. Information on jobs was available at retirement only. There was also insufficient detail on the classification of jobs by diesel exhaust exposure. The exposures could have been nonconcurrent or concurrent, but because the data are lacking, it is possible that the observed excess could be due to the effect of both coal dust and diesel fumes and not due to just one or the other. However, it should be noted that, so far, coal dust has not been demonstrated to be a pulmonary carcinogen in studies of coal miners, but lack of data on confounders such as asbestos and smoking makes interpretation of this study difficult. When three diesel exhaust exposure categories were examined for smoking-related diseases such as emphysema, laryngeal cancer, esophageal cancer, and buccal cancer, positive trends were observed, raising a possibility that the dose-response demonstrated for diesel exposure may have been due to smoking. The findings of this study are at best suggestive of diesel exhaust being a lung carcinogen.

The most definitive evidence for linking diesel exhaust exposure to lung cancer comes from the Garshick et al. (1988) railroad worker study conducted in the United States. Relative risks of 1.57 (95% CI = 1.19, 2.06) and 1.34 (95% CI = 1.02, 1.76) were found for ages 40 to 44 and 45 to 49, respectively, after the exclusion of workers exposed to asbestos. This study also found that the risk of lung cancer increased with increasing duration of employment. As this was a large cohort study with lengthy followup and adequate analysis, including dose response (based on duration of employment as a surrogate) as well as adjustment for other confounding factors such as asbestos, the observed association between increased lung cancer and exposure to diesel exhaust is more meaningful.

7.2.6.2. *Case-Control Studies of Lung Cancer*

Among the 10 lung cancer case-control studies reviewed in this chapter, only 2 studies did not find any increased risk of lung cancer. Lerchen et al. (1987) did not find any excess risk of lung cancer, after adjusting for age and smoking, for diesel fume exposure. The major limitation of this study was a lack of adequate exposure data derived from the job titles obtained from occupational histories. Next of kin provided the occupational histories for 50% of the cases that were not validated. The power of the study was small (analysis done on males only, 333 cases). Similarly, Boffeta et al. (1990) did not find any excess of lung cancer after adjusting for smoking and education. This study had a few methodological limitations. The lung cancer cases and controls were drawn from the ongoing study of tobacco-related diseases. It is interesting to note that the leading risk factor for lung cancer is cigarette smoking. The exposure was not measured. Instead, occupations were used as surrogates for exposure. Furthermore, there were very few individuals in the study who were exposed to diesel exhaust. On the other hand, statistically nonsignificant excess risks were observed for diesel exhaust exposure by Williams et al. (1977) in railroad workers (OR = 1.4) and truck drivers (OR = 1.34), by Hall and Wynder (1984) in workers who were exposed to diesel exhaust versus those who were not (OR = 1.4 and 1.7 with two different criteria), and by Damber and Larsson (1987) in professional drivers (OR = 1.2). These rates were adjusted for age and smoking. Both Williams et al. (1977) and Hall and Wynder (1984) had high nonparticipation rates of 47% and 36%, respectively. Therefore, the positive results found in these studies are underestimated at best. In addition, the self-reported exposures used in the study by Hall and Wynder (1984) were not validated. This study also had low power to detect excess risk of lung cancer for specific occupations.

The study by Benhamou et al. (1988), after adjusting for smoking, found significantly increased risks of lung cancer among French motor vehicle drivers (RR = 1.42) and transport equipment operators (RR = 1.35). The main limitation of the study was the inability to separate the exposures to diesel exhaust from those of gasoline exhaust because both motor vehicle drivers and transport equipment operators probably were exposed to the exhausts of both types of vehicles.

Hayes et al. (1989) combined data from three studies (conducted in three different states) to increase the power to detect an association between lung cancer and occupations with a high potential for exposure to diesel exhaust. They found that truck drivers employed for more than 10 years had a significantly increased risk of lung cancer (OR = 1.5, 95% CI = 1.1, 1.9). This study also found a significant trend of increasing risk of lung cancer with increasing duration of employment among truck drivers. The relative odds were computed by adjusting for birth cohort, smoking, and State of residence. The main limitation of this study is again the mixed exposures to

diesel and gasoline exhausts, because information on type of engine was lacking. Also, potential bias may have been introduced because the way in which the cause of death was ascertained for the selection of cases varied in the three studies. Furthermore, the methods used in these studies to classify occupational categories were different, probably leading to incompatibility of occupational categories.

The most convincing evidence comes from the Garshick et al. (1987) case-control study of railroad workers and the Steenland et al. (1990) case-control study of truck drivers in the Teamsters Union. Garshick et al. found that after adjustment for asbestos and smoking, the relative odds for continuous exposure were 1.39 (95% CI = 1.05, 1.83). Among the younger workers with longer diesel exhaust exposure, the risk of lung cancer increased with the duration of exposure after adjusting for asbestos and smoking. Even after the exclusion of recent diesel exhaust exposure (5 years before death), the relative odds increased to 1.43 (95% CI = 1.06, 1.94). This study appears to be a well-conducted and well-analyzed case-control study with reasonably good power. Potential confounders were controlled adequately, and interactions between diesel exhaust and other lung cancer risk factors were tested.

Steenland et al. (1990), on the other hand, created two separate work history files, one from Teamsters Union pension files and the other from next-of-kin interviews. Using duration of employment as a categorical variable and considering employment after 1959 (when presumed dieselization occurred) for long-haul drivers, the risk of lung cancer increased with increasing years of exposure. Using 1964 as the cutoff, a similar trend was observed for long-haul drivers. For short-haul drivers, the trend was positive with a 1959 cutoff but not when 1964 was used as the cutoff. For truck drivers who primarily drove diesel trucks and worked for 35 years, the relative odds were 1.89. The limitations of this study include possible misclassifications of exposure and smoking, lack of levels of diesel exposure, smaller nonexposed group, and insufficient latency period. Given these limitations, the findings of this study are probably underestimated.

Emmelin et al. (1993) in their Swedish dockworkers from 15 ports found increased relative odds of 6.8 (90% CI = 1.3 to 34.9). Intricate exposure matrices were created using three different variables, but no direct exposure measurement was done. Of 50 cases and 154 controls, only 6 individuals were nonsmokers. A strong interaction between smoking and diesel exhaust was observed in this study.

7.2.6.3. *Reviews and Meta-analyses of Lung Cancer*

Three summaries of studies concerned with the relationship of diesel exhaust exposure and lung cancer risk are reviewed. The HEI report is a narrative study of more than 35

epidemiologic studies (16 cohort and 19 case-control) of occupational exposure to diesel emissions published between 1957 and 1993. Control for smoking was identified in 15 studies. Six of the studies (17%) reported relative risk estimates less than 1, whereas 29 (83%) reported at least 1 relative risk, indicating a positive association. Twelve studies indicating a relative risk greater than 1 had 95% confidence intervals that excluded unity. These studies found that the evidence suggests that occupational exposure to diesel exhaust from diverse sources increases the rate of lung cancer by 20% to 40% in exposed workers generally, and to a greater extent among workers with prolonged exposure. They also found that the results are not explicable by confounding due to cigarette smoking of other known sources of bias.

Bhatia et al. (1998) identified 23 studies that met criteria for inclusion in the meta-analysis. The observed relative risk estimates were greater than 1 in 21 of these studies. The pooled relative risk weighted by study precision was 1.33 (95% CI= 1.24, 1.44), which indicated increased relative risk for lung cancer from occupational exposure to diesel exhaust. Subanalyses by study design (case-control and cohort studies) and by control for smoking produced results that did not differ from those of the overall pooled analysis. Cohort studies using internal comparisons showed higher relative risks than those using external comparisons.

Lipsett and Campleman (1999) identify 39 independent estimates of relative risk among 30 eligible studies of diesel exhaust and lung cancer published between 1975 and 1995. Pooled relative risks for all studies and for study subsets were estimated using a random effect model. Interstudy heterogeneity was also modeled and evaluated. A pooled smoking-adjusted relative risk was 1.47 (95% CI = 1.29, 1.67). Substantial heterogeneity was found in the pooled-risk estimates. Adjustment for confounding by smoking, having a lower likelihood of selection bias, and increased study power were all found to contribute to lower heterogeneity and increased pooled estimates of relative risk.

There is some variability in the conclusions of these summaries of the association of diesel exhaust and lung cancer. The three analyses find that smoking is unlikely to account for the observed effects, and all conclude that the data support a causal association between lung cancer and diesel exhaust exposure. On the other hand, Stober and Abel (1996), Muscat and Wynder (1995) and Cox (1997) call into question the assertions by Cohen and Higgins (1995), Bhatia et al. (1997), and Lipsett and Campleman (1999) that the associations seen for diesel exhaust and lung cancer are unlikely to be due to bias. They argue that methodologic problems are prevalent among the studies, especially in evaluation of diesel engine exposure and control of confounding by cigarette smoking. The conclusions of the two meta-analyses are based on magnitude of pooled relative risk estimates and evaluation of potential sources of heterogeneity in the estimates. Despite the statistical sophistication of the meta-analyses, the statistical models used cannot

compensate for deficiencies in the original studies and will remain biased to the extent that bias exists in the original studies.

It should be noted that a recent publication by Bruske-Hohlfeld et al. (1999) found a strong association between DE exposure and the occurrence of lung cancer. This pooled analysis of two case-control studies has a large sample size, is adjusted for smoking and asbestos exposures, and exposure to DE was estimated on the basis of job codes. This study is not critiqued in detail here but will be included when the document is finalized.

7.2.6.4. *Case-Control Studies of Bladder Cancer*

Of the seven bladder cancer case-control studies, four studies found increased risk in occupations with a high potential diesel exhaust exposure. A significantly increased risk of bladder cancer was found in Canadian railroad workers (RR = 9.0, 95% CI = 1.2, 349.5; Howe et al., 1980), truck drivers from New Hampshire and Vermont (OR = 2.9, $p<0.05$; Hoar and Hoover, 1985), and in Argentinean truck and railroad drivers (RR = 4.31, $p<0.002$; Iscovich et al., 1987). A positive trend with increasing employment as truck driver ($p=0.006$) was observed by Hoar and Hoover (1985) in their study of truck drivers from New Hampshire and Vermont. Significantly increased risks also were observed with increasing duration of employment of $\geq$20 years in truck drivers (OR = 12, $p=0.01$) and railroad workers (OR = 2.21, $p<0.05$; Steenland et al., 1987). No significant increased risk was found for any diesel-related occupations in studies by Wynder et al. (1985), Iyer et al. (1990), or Steineck et al. (1990). All these studies had several limitations, including inadequate characterization of diesel exhaust exposure, lack of validation of surrogate measures of exposure, and presence of other confounding factors (cigarette smoking, urinary retention, concentrated smoke within the truck cab, etc.); most of them had small sample sizes and none presented any latency analysis.

7.2.6.5. *Relevant Methodologic Issues*

Throughout this chapter, various methodologic limitations of individual studies have been discussed, such as small sample size, short followup period, lack of latency analysis, and lack of data on confounding variables. However, two of the major methodologic concerns in these studies are use of death certificates to determine cause of death and lack of data on cigarette smoking, which is a strong risk factor for both lung cancer and bladder cancer. Death certificates were used by all of the cohort mortality studies and some of the case-control studies of lung cancer and case-control studies of bladder cancer to determine cause of death. Use of death certificates could lead to misclassification bias. Studies of autopsies done between 1960 and 1971 demonstrated that lung cancer was overdiagnosed when compared with hospital discharge, with

no incidental cases found at autopsy (Rosenblatt et al., 1971). Schottenfeld et al. (1982) also found an overdiagnosis of lung cancer among autopsies conducted in 1977 and 1978. On the other hand, Percy et al. (1981) noted 95% concordance when comparing 10,000 lung cancer deaths observed in the Third National Cancer Survey from 1969 to 1971 (more than 90% were confirmed histologically) to death certificate coded cause of death. For bladder cancer, the concordance rate was 91%. These more recent findings suggest that the diagnosis of lung cancer as well as bladder cancer on death certificates is better than anticipated. Furthermore, an overdiagnosis of lung cancer or bladder cancer on death certificates would reduce the ability of the study to detect an effect of diesel exhaust exposure.

A persistent association of risk for lung cancer and diesel exhaust exposure is observed in more than 30 epidemiologic studies published over the past 40 years. Evaluation of whether this association can be attributed to a causal relation between diesel exhaust exposure and lung cancer requires careful consideration of whether chance, bias, or confounding might be likely alternative explanations.

Many of the studies provide confidence intervals for their estimates of excess risk or statistical tests, which indicate that it is unlikely that the individual study findings were due to random variation. The persistence of this association between diesel exhaust and lung cancer risk in so many studies indicates that the possibility is remote that the observed association in aggregate is due to chance. It is unlikely that chance alone accounts for the observed relation between diesel exhaust and lung cancer.

The excess risk is observed in both cohort and case-control designs, which contradicts the concern that a methodologic bias specifically characteristic of either design (e.g., recall bias) might account for the observed effect. Selection bias is certainly present in some of the occupational cohort studies that use external population data in estimating relative risks, but this form of selection bias (a healthy worker effect) would only obscure, rather than spuriously produce, an association between diesel exhaust and lung cancer. Several occupational epidemiologic studies that use more appropriate data for their estimates are available. Selection biases may be operating in some case-control studies, but it is not obvious how such a bias could be sufficiently uniform in effect, prevalent, and strong enough to lead to the persistent association seen in the aggregate data. Given the variety of designs used in studying the diesel exhaust and lung cancer association and the number of studies in different populations, it is unlikely that routinely studying noncomparable groups is an explanation for the persistent association seen. Exposure information bias is certainly a problem for almost all of the studies concerned. Detailed and reliable individual-level data on diesel exhaust exposure for the period of time relevant to the induction of lung cancer are not available and are difficult to obtain. Generally, the only

information from which diesel exposure can be inferred is occupational data, which is a poor surrogate for the true underlying exposure distribution. Study endpoints are frequently mortality data taken from death certificate information, which is frequently inaccurate and often does not fully characterize the lung cancer incidence experience of the population in question. Using inaccurate surrogates for lung cancer incidence and for diesel exposure can lead to substantial bias, and these shortcomings are endemic in the field. In most cases these shortcomings will lead to misclassification of exposure and of outcome, which is nondifferential. Nondifferential misclassification of exposure and/or outcome can bias estimates of a diesel exhaust–lung cancer association, if one exists, toward the null; but it is unlikely that such misclassification would produce a spurious estimate in any one study. It is even more unlikely that it would bias a sufficient number of studies in a uniform direction to account for the persistent aggregate association observed.

All the cohort studies considered for this report are retrospective mortality studies. Smoking history is usually difficult to obtain in such instances. The smoking histories obtained from surrogates (next of kin, either spouse or offspring) were found to be accurate by Lerchen and Samet (1986) and McLaughlin et al. (1987). Lerchen and Samet did not detect any consistent bias in the report of cigarette consumption. In contrast, overreporting of cigarette smoking by surrogates was observed by Rogot and Reid (1975), Kolonel et al., (1977), and Humble et al. (1984). Kolonel et al. found that the age at which an individual started smoking was reported within 4 years of actual age 84% of the time. These studies indicate that surrogates were able to provide fairly credible information on the smoking habits of the study subjects. If the surrogates of the cases were more likely to overreport cigarette smoking compared with the controls, then it might be harder to find an effect of diesel exhaust because most of the increase in lung cancer would be attributed to smoking rather than to the effect of exposure to diesel exhaust.

Many studies do not adjust for tobacco smoke exposure. These studies are correctly dismissed as not contributing to the body of information suitable for causal inference. Several studies do attempt to adjust for smoking. Sometimes the data are aggregate data and the methods used for adjustment are indirect and rely on critical and unverifiable assumptions for effective adjustment (Pfluger, 1994). Frequently, individual-level data are used to adjust estimates of effect by conventional methods. Usually, these data are not a careful, detailed, and thorough assessment of smoking behavior. Generally the classification of smoking behavior is crude (smoker vs. nonsmoker) and cannot be considered to fully characterize actual exposure. Given these shortcomings, a possibility remains that the statistical adjustment for smoking is not completely effective, and residual confounding by smoking may persist to bias the measure of the diesel exhaust-lung cancer association.

7.2.6.6. *Criteria of Causal Inference*

In most situations, epidemiologic data are used to delineate the causality of certain health effects. Several cancers have been causally associated with exposure to agents for which there is no direct biological evidence. Insufficient knowledge about the biological basis for diseases in humans makes it difficult to identify exposure to an agent as causal, particularly for malignant diseases when the exposure was in the distant past. Consequently, epidemiologists and biologists have provided a set of criteria that define a causal relationship between exposure and the health outcome. A causal interpretation is enhanced for studies that meet these criteria. None of these criteria actually proves causality; actual proof is rarely attainable when dealing with environmental carcinogens. None of these criteria should be considered either necessary (except temporality of exposure) or sufficient in itself. The absence of any one or even several of these criteria does not prevent a causal interpretation. However, if more criteria apply, this provides more credible evidence for causality.

Thus, applying the criteria of causal inference to the seven cohort mortality and eight case-control studies in which risk of lung cancer was assessed resulted in the following:

- **Temporality:** There is a temporality of exposure to diesel exhaust prior to the occurrence of lung cancer in every cohort and case-control study.

- **Strength of association:** The strength of association between exposure and the occurrence of lung cancer in the cohort studies showed a 30% to 57% higher risk among exposed persons as compared with nonexposed (Howe et al., 1983; Wong et al., 1985; Boffetta and Stellman, 1988; Garshick et al., 1988). In case-control studies, the risk varied from 20% to 89% higher among exposed compared with nonexposed (Williams et al., 1977; Hall and Wynder, 1984; Damber and Larsson, 1987; Garshick et al., 1987; Benhamou et al., 1988; Hayes et al., 1989; Steenland et al., 1990; Gustavsson et al., 1990; Emmelin et al., 1993). Some of these studies did adjust for the confounding effects of smoking, asbestos, and other exposures. Furthermore, a recent publication by HEI (1995) demonstrates this strength of association in graphic presentation (Figures 7-3 and 7-4). Meta-analyses by Bhatia et al. and Lipsett et al. also show the pooled estimated RR of 1.33 and 1.47, respectively. Although the studies had smaller increases in lung cancer risk and only some of the studies considered by HEI (1995) are considered in this chapter, it demonstrates the lung cancer excesses consistently all across the various populations.

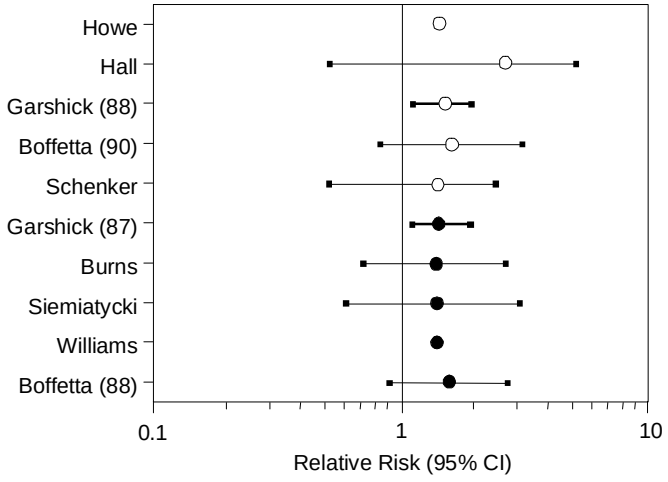


Figure 7-3. Lung cancer and exposure to diesel exhaust in railroad workers. ● = Relative risk adjusted for cigarette smoking; ○ = relative risk not adjusted for cigarette smoking. For the two studies by Howe and Williams, confidence intervals were not reported and could not be calculated.

Source: HEI, 1995.

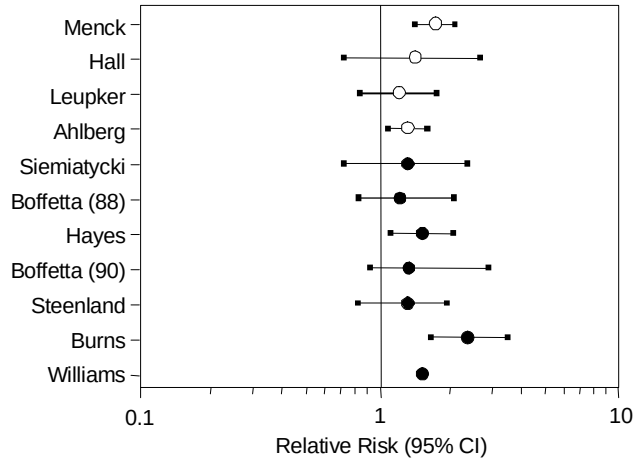


Figure 7-4. Lung cancer and exposure to diesel exhaust in truck drivers. ● = Relative risk adjusted for cigarette smoking; ○ = relative risk not adjusted for cigarette smoking. For the study by Williams, confidence intervals were not reported and could not be calulated. For the Steenland study, the data were gathered from union reports of long-haul truck drivers; for the Boffetta (1988) study, the data were self-reported by diesel truck drivers; and for the Siemiatycki study, they were self-reported by heavy-duty truck drivers (personal communication).

Source: HEI, 1995.

- **Consistency:** Several cohort and case-control (including one nested case-control) studies of lung cancer conducted in several populations in the United States and Europe consistently found the same effect (i.e., lung cancer).

- **Specificity:** All of the above-mentioned studies found the same effect (i.e., lung cancer).

- **Biological gradient:** The biological gradient, which refers to the dose-response relationship, was observed in the cohorts of Canadian railway workers (Howe et al., 1983), heavy bulldozer operators (Wong et al., 1985), and truck drivers who had enrolled in the American Cancer Society's prospective mortality study (Boffetta and Stellman, 1988). In the case-control studies, a dose response was observed in railroad workers (Garshick et al., 1988; Hayes et al., 1989; Steenland et al., 1990). Although other studies failed to observe a dose response, these studies were methodologically limited due to confounding by other exposures and lack of either quantitative data on exposure or surrogate data on dose.

- **Biological plausibility:** Because diesel exhaust consists of a carbon core particle with surface layers of organics and gases, the tumorigenic activity may reside in one, some, or all of these components. As explained in Chapter 9, there is clear evidence that the organic constituents have the capacity to interact with DNA and give rise to mutations, chromosomal aberrations, and cell transformations, all well-established steps in the process of carcinogenesis. Furthermore, these organic chemicals include a variety of polycyclic aromatic hydrocarbons and nitroaromatics, many of which are known to be pulmonary carcinogens. Alternatively, Vostal (1986) suggests that "diesel" particles themselves induce lung cancer, most likely via an epigenetic mechanism, if they are present at sufficiently high doses. This makes a convincing argument for biological plausibility of lung cancer occurrence under some condition of exposure.

When the same causal inference criteria were applied to the seven case-control studies in which risk of bladder cancer was assessed, the results were:

- **Temporality:** There is temporality of exposure to diesel exhaust prior to the occurrence of bladder cancer.

- **Strength of association:** The relative odds of getting bladder cancer among exposed compared with nonexposed ranged from 2 to 12 times higher (Howe et al., 1980; Hoar and Hoover, 1985; Iscovich et al., 1987; Steenland et al., 1987). None of these studies adjusted for other confounding effects such as cigarette smoking, exposures to other chemicals, or urinary retention.

- **Consistency:** Four out of seven bladder case-control studies conducted in the United States and abroad found increased relative odds of bladder cancer in the exposed population. None of the cohort studies showed increased bladder cancer mortality; however, people rarely die from bladder cancer, so bladder cancer excess is unlikely to be detected in mortality studies.

- **Specificity:** Four out of seven case-control studies found an excess of bladder cancer. The specificity criterion, per se, does not apply in this particular instance because these are case-control studies.

- **Biological gradient:** Dose response was observed in two out of four studies showing increasing relative odds with increasing length of employment (Hoar and Hoover, 1985; Steenland et al., 1987).

- **Biological plausibility:** It has been demonstrated that motor exhaust emissions contain PAHs and nitro-PAHs (Stenberg et al., 1983; Rosenkranz and Mermelstein, 1983). There is some evidence that nitro-PAHs may be responsible for the induction of human bladder cancer. Nitro-PAHs can be metabolized to aromatic amine derivatives, and some of these agents are known to be capable of inducing urinary bladder cancer (Clayson and Garner, 1976). Furthermore, 1-nitropyrene (1-NP) has been reported to be carcinogenic in the rat mammary gland (Hirose et al., 1984); the structurally related 4-aminobiphenyl, which induces bladder cancer in humans, also induces mammary gland tumors in rats (Hirose et al., 1984). Although the applicability of these experimental results to humans is unknown, the laboratory evidence certainly suggests the biological plausibility of diesel exhaust to be a urinary bladder carcinogen.

In summary, although some of the causality inference criteria do apply to bladder cancer, the evidence for bladder cancer in populations exposed to diesel exhaust is inadequate. On the other hand, all the causality inference criteria apply well to lung cancer. An excess risk of lung cancer was observed in several cohort and case-control studies. A recent meta-analysis shows the consistency of elevated risks in 23 of 29 diesel exposure epidemiologic studies, with statistically significant relative risks of 1.33 (Bhatia et al., 1998). Lipsett et al. (1999) also found a pooled estimate RR of 1.47 after adjusting for smoking. However, because of lack of actual data on exposure to diesel exhaust in these studies and other subtle methodologic limitations, the human evidence falls just short of being sufficient to call diesel exhaust a human carcinogen.

7.3. CARCINOGENICITY OF DIESEL EMISSIONS IN LABORATORY ANIMALS

This chapter summarizes studies that assess the carcinogenic potential of diesel exhaust in laboratory animals. The first portion of this chapter summarizes results of inhalation studies. Experimental protocols for the inhalation studies typically consisted of exposure (usually chronic) to diluted exhaust in whole-body exposure chambers using rats, mice, and hamsters as model species. Some of these studies used both filtered (free of particulate matter) diesel exhaust and unfiltered (whole) diesel exhaust to differentiate gaseous-phase effects from effects induced by DPM and its adsorbed components. Other studies were designed to evaluate the relative

importance of the carbon core of the diesel particle versus that of particle-adsorbed compounds. Finally, a number of exposures were carried out to determine the combined effect of inhaled diesel exhaust and tumor initiators, tumor promoters, or co-carcinogens.

Particulate matter concentrations in the diesel exhaust used in these studies ranged from 0.1 to 12 mg/m^3. In this chapter, any indication of statistical significance implies that $p \leq 0.05$ was reported in the reviewed publications. The experimental protocols and exposure atmosphere characterizations are not described in detail here but may be found in Appendix A. A summary of the animal inhalation carcinogenicity studies and their results is presented in Table 7-4.

Results of lung implantation and intratracheal instillation studies of whole diesel particles, extracted diesel particles, and particle extracts are reported in Section 7.3.3 and in Tables 7-6 and 7-7. Studies destined to assess the carcinogenic effects of DPM as well as solvent extracts of DPM following subcutaneous (s.c.) injection, intraperitoneal (i.p.) injection, or intratracheal (itr.) instillation in rodents are summarized in Section 7.3.5. Individual chemicals present in the gaseous phase or adsorbed to the particle surface were not included in this review because assessments of those of likely concern (i.e., formaldehyde, acetaldehyde, benzene, PAHs) have been published elsewhere (U.S. EPA, 1993).

7.3.1. Inhalation Studies (Whole Diesel Exhaust)

7.3.1.1. *Rat Studies*

The potential carcinogenicity of inhaled diesel exhaust was first evaluated by Karagianes et al. (1981). Male Wistar rats (40 per group) were exposed to room air or diesel engine exhaust diluted to a DPM concentration of 8.3 (± 2.0) mg/m^3, 6 hr/day, 5 days/week for up to 20 mo. The animals were exposed in 3,000 liter plexiglass chambers. Airflow was equal to 50 liters per minute. Chamber temperatures were maintained between 25° and 26.5 °C. Relative humidity ranged form 45% to 80%. Exposures were carried out during the daytime. The exhaust-generating system and exposure atmosphere characteristics are presented in Appendix A. The type of engine used (3-cylinder, 43 bhp diesel) is normally used in mining situations and was

Table 7-4. Summary of animal inhalation carcinogenicity studies

Study	Species/ strain	Sex/total number	Exposure atmosphere	Particle concentration (mg/m^3)	Other treatment	Exposure protocol	Post-exposure observation	Tumor type and incidence (%)[a]	Comments
								Adenomas	
Karagianes et al. (1981)	Rat/Wistar	M, 40	Clean air	0	None	6 hr/day, 5 days/week, for up to 20 mo	NA	0/6 (0)	
		M, 40	Whole exhaust	8.3	None			1/6 (16.6)	
								Broncho-alveolar carcinoma	
Kaplan et al. (1983) White et al. (1983)	Rat/F344	M, 30	Clean air	0	None	20 hr/day, 7 days/week, for up to 15 mo	8 mo	0/30 (0)	
		M, 30	Whole exhaust	0.25	None		8 mo	1/30 (3.3)	
		M, 30	Whole exhaust	0.75	None		8 mo	3/30 (10.0)	
		M, 30	Whole exhaust	1.5	None		8 mo	1/30 (3.3)	
								Adenomas / Carcinomas / Squamous cell tumors / All tumors	
Heinrich et al. (1986a,b) Mohr et al. (1986)	Rat/ Wistar	F, 96	Clean air	0	None	19 hr/day, 5 days/week for up to 35 mo	NA	0/96 (0) / 0/96 (0) / 0/96 (0) / 0/96 (0)	
		F, 92	Filtered exhaust	0	None			0/92 (0) / 0/92 (0) / 0/92 (0) / 0/92 (0)	
		F, 95	Whole exhaust	4.0	None			8/95 (8.4) / 0/95 (0) / 9/95 (9.4) / 17/95 (17.8)[c]	
								Adenomas / Adenocarcinoma and adenosquamous carcinoma / Large cell and squamous cell carcinomas / All tumors	
Iwai et al. (1986)	Rat/F344	F, 24	Clean air	0	None	8 hr/day, 7 days/week, for 24 mo	NA	1/22 (4.5) / 0/22 (0) / 0/22 (0) / 1/22 (4.5)[f]	
		F, 24	Filtered exhaust	0	None			0/16 (0) / 0/16 (0) / 0/16 (0) / 0/16 (0)	
		F, 24	Whole exhaust	4.9	None			3/19 (0) / 3/19 (15.8) / 2/19 (10.5) / 8/19 (42.1)[c,g]	
								Adenoma / Carcinoma	
Takemoto et al. (1986)	Rat/F344	F, 12	Clean air	0	None	4 hr/day, 4 days/week, 18-24 mo	NA	0/12 (0) / 0/12 (0)	
		F, 21	Clean air	0	DIPN[h]			10/21 (47.6) / 4/21 (19)	
		F, 15	Whole exhaust	2-4	None			0/15 (0) / 0/15 (0)	
		F, 18	Whole exhaust	2-4	DIPN[h]			12/18 (66.7) / 7/18 (38.9)	
								Adenomas / Adenocarcinoma + squamous cell carcinoma / Squamous cysts / All tumors	
Mauderly et al. (1987)	Rat/F344	M + F, 230[b]	Clean air	0	None	7 hr/day, 5 days/week up to 30 mo	NA	(0) / (0.9) / (0) / (0.9)	
		M + F, 223	Whole exhaust	0.35	None			(0) / (1.3) / (0) / (1.3)	
		M + F, 221	Whole exhaust	3.5	None			(2.3) / (0.5) / (0.9) / (3.6)[c]	
		M + F, 227	Whole exhaust	7.1	None			(0.4) / (7.5) / (4.9) / (12.8)[c]	

Table 7-4. Summary of animal inhalation carcinogenicity studies (continued)

Study	Species/ strain	Sex/total number	Exposure atmosphere	Particle concentration (mg/m^3)	Other treatment	Exposure protocol	Post-exposure observation	Tumor type and incidence (%)[a]				Comments
								Adenomas	Adenosquamous carcinomas	Squamous cell carcinomas	All tumors	
Ishinishi et al. (1988a)	Rat/F344	M + F, 123	Clean air	0	None	16 hr/day,	NA	0/123 (0)	1/123 (0.8)	0/123 (0)	1/123 (0.8)	
		M + F, 123	Whole exhaust	0.5	None	6 days/week,		0/123 (0)	0/123 (0)	1/123 (0.8)	1/123 (0.8)	
		M + F, 125	Whole exhaust	1.0	None	for up to		0/125 (0)	0/125 (0)	0/125 (0)	0/125 (0)	
Heavy-duty		M + F, 123	Whole exhaust	1.8	None	30 mo		0/123 (0)	4/123 (3.3)	0/123 (0)	4/123 (3.3)	
engine		M + F, 124	Whole exhaust	3.7	None			0/124 (0)	6/124 (4.8)	2/124 (1.6)	8/124 (6.5)[c]	
								Adenomas	Carcinomas	All tumors		
Ishinishi et al. (1988a)	Rat/F344	NS, 5	Whole exhaust	0.1	None	16 hr/day,	6 mo	0/5 (0)	0/5 (0)	0/5 (0)		
		NS, 8	Whole exhaust	0.1	None	6 days/week,	12 mo	0/8 (0)	0/8 (0)	0/8 (0)		
		NS, 11	Whole exhaust	0.1	None	for 12 mo	18 mo	0/11 (0)	0/11 (0)	0/11 (0)		
Light duty		NS, 5	Whole exhaust	1.1	None		6 mo	0/5 (0)	0/5 (0)	0/5 (0)		
		NS, 9	Whole exhaust	1.1	None		12 mo	0/9 (0)	0/9 (0)	0/9 (0)		
		NS, 11	Whole exhaust	1.1	None		18 mo	0/11 (0)	0/11 (0)	0/11 (0)		
Heavy duty		NS, 5	Whole exhaust	0.5	None	16 hr/day,	6 mo	0/5 (0)	0/5 (0)	0/5 (0)		
		NS, 9	Whole exhaust	0.5	None	6 days/week,	12 mo	0/9 (0)	0/9 (0)	0/9 (0)		
		NS 11	Whole exhaust	0.5	None	for 12 mo	18 mo	0/11 (0)	0/11 (0)	0/11 (0)		
		NS, 5	Whole exhaust	1.8	None		6 mo	0/5 (0)	0/5 (0)	0/11 (0)		
		NS, 6	Whole exhaust	1.8	None		12 mo	0/6 (0)	0/6 (0)	0/6 (0)		
		NS, 13	Whole exhaust	1.8	None		18 mo	0/13 (0)	1/13 (0)	1/13 (0)		
									Primary lung tumors			
Brightwell et al. (1989)	Rat/344	M + F, 260	Clean air	0	None	16 hr/day,	NA		3/260 (1.2)			Tumor incidence for all rats dying or sacrificed
		M + F, 144	Filtered exhaust (medium exposure)	0	None	5 days/week, for 24 mo			0/144 (0)			
		M + F, 143	Filtered exhaust (high exposure)	0	None				0/143 (0)			
		M + F, 143	Whole exhaust	0.7	None				1/143 (0.7)			♀ 24/25 (96%) after 24 mo
		M + F, 144	Whole exhaust	2.2	None				14/144 (9.7)[c]			♂ 12/27 (44%) after 24 mo
		M + F, 143	Whole exhaust	6.6	None				55/143 (38.5)[c]			

Table 7-4. Summary of animal inhalation carcinogenicity studies (continued)

Study	Species/ strain	Sex/total number	Exposure atmosphere	Particle concentration (mg/m^3)	Other treatment	Exposure protocol	Post-exposure observation	Tumor type and incidence (%)[a]				Comments
										Squamous cell carcinoma	All lung tumors	
Henrich et al. (1989a)	Rat/Wistar	F, NS	Clean air	0	DPN[d]	19 hr/day, 5 days/week for 24 to 30 mo	NA			(4.4)	(84.8)	
		F, NS	Whole exhaust	4.2	DPN[d]					(46.8)[c]	(83.0)	
		F, NS	Filtered exhaust	0	DPN[d]					(4.4)	(67.4)	
		F, NS	Clean air	0	DPN[e]					(16.7)	(93.8)	
		F, NS	Whole exhaust	4.2	DPN[e]					(31.3)[c]	(89.6)	
		F, NS	Filtered exhaust	0	DPN[e]					(14.6)	(89.6)	
Lewis et al. (1989)	Rat/F344	M + F, 288[n]	Clean air	0	None	7 hr/day, 5 days/week, 24 mo	NA	No tumors			0/192 (0)	
			Whole exhaust	2.0	None						0/192 (0)	
								Adenosquamous carcinomas	Squamous cell carcinomas	All tumors		
Takaki et al. (1989) Light-duty engine	Rat/F344	M + F, 123	Clean air	0	None	16 hr/day, 6 days/week, for up to 30 mo	NA	1/23 (0.8)	2/123 (1.6)	1/23 (0.8)	4/123 (3.3)	
		M + F, 123	Whole exhaust	0.1	None			1/23 (0.8)	1/23 (0.8)	1/23 (0.8)	3/123 (2.4)	
		M + F, 125	Whole exhaust	0.4	None			1/25 (0.8)	0/125 (0)	0/125 (0)	1/125 (0.8)	
		M + F, 123	Whole exhaust	1.1	None			0/23 (0)	5/123 (4.1)	0/123 (0)	5/123 (4.1)	
		M + F, 124	Whole exhaust	2.3	None			1/24 (8.1)	2/124 (1.6)	0/124 (0)	3/124 (2.4)	
								Adenomas	Adenocarcinomas	Squamous cell carcinomas	Benign squamous cell tumors	
Heinrich et al. (1995)	Rat/Wistar	F, 220	Clean air	0	None	18 hr/day, 5 days/week, for up to 24 mo	6 mo	0/217 (0)	1/217 (<1)	0/217 (0)	0/217 (0)	
		F, 200	Whole exhaust	0.8	None			0/198 (0)	0/198 (0)	0/198 (0)	0/198 (0)	
		F, 200	Whole exhaust	2.5	None			2/200 (1)	1/200 (<1)	0/200 (0)	7/200 (3.5)	
		F, 100	Whole exhaust	7.0	None			4/100 (4)	4/100 (4)	2/100 (2)	14/100 (14)	Tumor incidences after 30 mo
		F, 100	Carbon black	11.6	None			13/100 (13)	13/100 (13)	4/100 (4)	20/100 (20)	
		F, 100	TiO_2	10.0	None			4/100 (4)	13/100 (13)	3/100 (3)	20/100 (20)	
								Adenomas	Adenocarcinomas	Squamous cell carcinoma	Adeno-squamous carcinoma	Other neoplasms
Nikula et al. (1995)	Rat/F344	M + F, 214[b]	Clean air	0	None	16 hr/day, 5 days/week for up to 24 mo	6 weeks	1/214 (<1)	1/214 (<1)	1/214 (<1)	0/214 (0)	0/214 (0)
		M + F, 210	Whole exhaust	2.5	None			7/210 (3)	4/210 (2)	3/210 (1)	0/210 (0)	0/210 (0)
		M + F, 212	Whole exhaust	6.5	None			23/212 (11)	22/212 (10)	3/212 (1)	1/212 (<1)	0/212 (0)
		M + F, 213	Carbon black	2.5	None			3/213 (1)	7/213 (3)	0/213 (0)	0/213 (0)	1/213 (<1)
		M + F, 211	Carbon black	6.5	None			13/211 (6)	21/211 (10)	3/211 (1)	2/211 (<1)	0/211 (0)
Iwai et al. (1997)	F/344	121, F	Clean air	0	None	NA	NA	5/121(4%) type not stated				Cumulative exposure dose ranged from 154-274 mg/cum
		108, F	Filtered air	0	None	48-56 hr/day	6 mo	2/108(4%) type not stated				
		153, F	Whole exhaust	3.2-9.4	None	48-56 hr/day	6 mo	53/153(35%) 61.3% adenoma, 25.8% adenocarcinoma, 2.2% benign squamous cell tumor, 7.5% squamous cell carcinoma, 3.2% adenosquamous carcinoma				

Table 7-4. Summary of animal inhalation carcinogenicity studies (continued)

Study	Species/ strain	Sex/total number	Exposure atmosphere	Particle concentration (mg/m^3)	Other treatment	Exposure protocol	Post-exposure observation	Tumor type and incidence (%)[a]	Comments
Orthoefer et al. (1981) (Pepelko and Peirano, 1983)	Mouse/ Strong A	M, 25	Clean air	0	None	20 hr/day, 7 days/week, for 7 weeks		3/22 (13.6)	0.13 tumors/ mouse
			Whole exhaust	6.4	None		26 weeks	7/19 (36.8)	0.63 tumors/ mouse
			Whole exhaust	6.4	UV irradiated		26 weeks	6/22 (27.3)	0.27 tumors/ mouse
								Lung tumors	
	Mouse/ Jackson A	M + F, 40	Clean air	0	None	20 hr/day, 7 days/week, for 8 weeks	8 weeks	16/36 (44.4)	0.5 tumors/ mouse
		M + F, 40	Whole exhaust	6.4	None		8 weeks	11/34 (32.3)	0.4 tumors/ mouse
	Mouse/ Jackson A	F, 60	Clean air	0	None	20 hr/day, 7 days/week, for approx. 7 mo.		4/58 (6.9)	0.09 tumors/ mouse
		F, 60	Clean air	0	Urethan[l]			9/52 (17.3)	0.25 tumors/ mouse
		F, 60	Whole exhaust	6.4	None			14/56 (25.0)	0.32 tumors/ mouse
		F, 60	Whole exhaust	6.4	Urethan[k]			22/59 (37.3)	0.39 tumors/ mouse
		M, 429	Clean air	0	None			73/403 (18.0)	0.23 tumors/ mouse
		M, 430	Whole exhaust	6.4	None			66/368 (17.9)	0.20 tumors/ mouse
Kaplan et al. (1982)	Mouse A/J	M, 458	Clean air	0	None	20 hr/day, 7 days/week, for 3 mo	6 mo	Pulmonary adenomas	
		M, 18	Clean air	0	Urethan[k]			144/458 (31.4)	
		M, 485	Whole exhaust	1.5	None			18/18 (100)	
								165/485 (34.2)	
								Pulmonary adenoma	

Table 7-4. Summary of animal inhalation carcinogenicity studies (continued)

Study	Species/ strain	Sex/total number	Exposure atmosphere	Particle concentration (mg/m^3)	Other treatment	Exposure protocol	Post-exposure observation	Tumor type and incidence (%)[a]				Comments
Kaplan et al. (1983) White et al. (1983)	Mouse/ A/ J	M, 388 M, 388 M, 399 M, 396	Clean air Whole exhaust Whole exhaust Whole exhaust	0 0.25 0.75 1.5	None None None None	20 hr/day, 7 days/week, for up to 8 mo	NA	130/388 (33.5) 131/388 (33.8) 109/399 (27.3) 99/396 (25.0)				
Pepelko and Peirano (1983)	Mouse/ Sencar	M + F, 260	Clean air Clean air Clean air Whole exhaust Whole exhaust Whole exhaust	0 0 0 12 12 12	None BHT[l] Urethan[k] None BHT[l] Urethan[l]	Continuous for 15 mo	NA	Adenomas (5.1) (12.2) (8.1) (10.2)[c] (5.4) (8.7)	Carcinomas (0.5) (1.7) (0.9) (1.0) (2.7) (2.6)	All tumors (5.6) (2.8) (9.0) (11.2)[c] (8.1) (11.2)		
Pepelko and Peirano (1983)	Mouse/ Strain A	M + F, 90	Clean air Clean air Whole exhaust Whole exhaust Clean air Whole exhaust	0 0 12 12 0 12	None Exposure (darkness) Exposure (darkness) Urethan[m] Urethan[m]		NA	All tumors 21/87 (24) 59/237 (24.9) 10/80 12.5) 22/250 (0.10) 66/75 (88) 42/75 (0.95)				0.29 tumors/ mouse 0.27 tumors/ mouse 0.14 0.10 2.80 0.95
Heinrich et al. (1986a,b)	Mouse/ NMRI	M + F, 84 M + F, 93 M + F, 76	Clean air Filtered exhaust Whole exhaust	0 0 4.0	None None None	19 hr/day, 5 days/week for up to 30 mo	NA	Adenomas 9/84 (11) 11/93 (12) 11/76 (15)	Adenocarcinoma 2/84 (2) 18/93 (19)[c] 13/76 (17)[c]	Squamous cell tumors — — —	All tumors 11/84 (13) 29/93 (31)[c] 24/76 (32)[c]	
Takemoto et al. (1986)	Mouse/ IRC	M + F, 45 M + F, 69	Clean air Whole exhaust	0 2-4	None None	4 hr/day, 4 days/week, for 19-28 mo	NA		Adenoma	Adenocarcinoma		
	Mouse/ C57BL	M + F, 12 M + F, 38	Clean air Whole exhaust	0 2-4	None None	4 hr/day, 4 days/week for 19-28 mo	NA		3/45 (6.7) 6/69 (8.7)	1/45 (2.2) 3/69 (4.3)		

Table 7-4. Summary of animal inhalation carcinogenicity studies (continued)

Study	Species/ strain	Sex/total number	Exposure atmosphere	Particle concentration (mg/m^3)	Other treatment	Exposure protocol	Post-exposure observation	Tumor type and incidence (%)[a]				Comments
Heinrich et al. (1995)	Mouse/ C57BL/ 6N	F, 120	Clean air	0	None	18 hr/day, 5 days/week, for up to 21 mo	6 mo	1/12 (8.3) 8/38 (21.1)	0/12 (0) 3/38 (7.9)			5.1% tumor rate
		F, 120	Whole exhaust	4.5	None							8.5% tumor rate
		F, 120	Particle-free exhaust	0	None							3.5% tumor rate
	Mouse/ NMRI	F, 120	Clean air	0	None	18 hr/day, 5 days/week for up to 13.5 mo	9.5 mo	Adenomas	Adenocarcinomas			
		F, 120	Whole exhaust	4.5	None			(25)	(15.4)			
			Carbon black	11.6	None			(21.8)	(15.4)			
			TiO_2	10	None			(11.3)	(10)			
								(11.3)	(2.5)			
	Mouse/ NMRI	F, 120	Clean air	0	None	18 hr/day, 5 days/week, 23 mo	None	(25)	(8.8)			
		F, 120	Whole exhaust	4.5	None			(18.3)	(5.0)			
		F, 120	Particle-free exhaust	0	None			(31.7)	(15)			
								Multiple adenomas	Multiple carcinomas	Adenomas/ carcinoma	Alveolar/ bronchiolar adenoma	Alveolar/ bronchiolar carcinoma
Mauderly et al. (1996)	Mouse/ CD-1	M + F, 157[b]	Clean air	0	None	7 hr/day, 5 days/week, for up to 24 mo	None	1/157 (0.6)	2/157 (1.3)	1/157 (0.6)	10/157 (6.4)	7/157 (4.5)
		M + F, 171	Whole exhaust	0.35	None			2/171 (1.2)	1/171 (0.6)	1/171 (0.6)	16/171 (9.4)	5/171 (2.9)
		M + F, 155	Whole exhaust	3.5	None			0/155 (0)	1/155 (0.6)	0/155 (0)	8/155 (5.2)	6/155 (3.9)
		M + F, 186	Whole exhaust	7.0	None			0/186 (0)	0/186 (0)	0/186 (0)	10/186 (5.4)	4/186 (2.2)

Table 7-4. Summary of animal inhalation carcinogenicity studies (continued)

Study	Species/ strain	Sex/total number	Exposure atmosphere	Particle concentration (mg/m^3)	Other treatment	Exposure protocol	Post-exposure observation	Tumor type and incidence (%)[a]				Comments
								Adenomas	Adenocarcinoma	Squamous cell tumors	All tumors	
Heinrich et al. (1986a,b)	Hamster/ Syrian	M + F, 96	Clean air	0	None	19 hr/day		0/96(0)	0/96(0)	0/96	0/96(0)	
		M + F, 96	Filtered exhaust	0	None	5 days/week for up to		0/96(0)	0/96(0)	0/96	0/96(0)	
		M + F, 96	Whole exhaust	4.0	None	30 mo	NA	0/96(0)	0/96(0)	0/96	0/96(0)	
									Primary lung tumors			
Brightwell et al. (1989)	Hamster/ Syrian Golden	M + F,	Clean air	0	None	16 hr/day,	NA		7/202 (3.5)			Respiratory tract tumors not related to exhaust exposure for any of the groups
		M + F, 202	Clean air	0	DEN[j]	5 days/week,			4/104 (3.8)			
		M + F, 104	Filtered exhaust (medium dose)	0	DEN[j]	for 24 mo			9/104 (8.7)			
		M + F, 104	Filtered exhaust (high dose)	0	DEN[j]				2/101 (2.0)			
		M + F, 101	Whole exhaust	0.7	DEN[j]				6/102 (5.9)			
		M + F, 102	Whole exhaust	2.2	DEN[j]				4/101 (3.9)			
		M + F, 101	Whole exhaust	6.6	DEN[j]				1/204 (0.5)			
		M + F, 204	Filtered exhaust (high dose)	0	None				0/203 (0)			
		M + F, 203	Whole exhaust	6.6	None							

[a]Table values indicate number with tumors/number examined (% animals with tumors).
[b]Number of animals examined for tumors.
[c]Significantly different from clean air controls.
[d]Dipentylnitrosamine; 6.25 mg/kg/week s.c. during first 25 weeks of exposure.
[e]Dipentylnitrasamine; 12.5 mg/kg/week s.c. during first 25 weeks of exposure.
[f]Splenic lymphomas also detected in controls (8.3%), filtered exhaust group (37.5%) and whole exhaust group (25%).
[g]5.3% incidence of large cell carcinomas.
[h]1 g/kg, i.p. 1/week for 3 weeks starting 1 mo into exposure.
[i]Includes adenomas, squamous cell carcinomas, adenocarcinomas, adenosquamous cell carcinoma, and mesotheliomas.
[j]4.5 mg/diethylnitrosamine (DEN)/kg, s.c., 3 days prior to start of inhalation exposure.
[k]Single i.p. dose 1 mg/kg at start of exposure.
[l]Butylated hydroxytoluene 300 mg/kg, i.p. for week 1, 83 mg/kg for week 2, and 150 mg/kg for weeks 3 to 52.
[m]12 $mg/m^3$from 12 weeks of age to termination of exposure. Prior exposure (in utero) and of parents was 6 mg/m^3.
[n]120-121 males and 71-72 females examined histologically.
[o]Not all animals were exposed for full term, at least 10 males were killed at 3, 6, and 12 mo of exposure.
NS = Not specified.

NA = Not applicable.

connected to an electric generator and operated at varying loads and speeds to simulate operating conditions in an occupational situation. To control the CO concentration at 50 ppm, the exhaust was diluted 35:1 with clean air. Six rats per group were sacrificed after 4, 8, 16, and 20 mo exposure for gross necropsy and histopathological examination.

The only tumor detected was a bronchiolar adenoma in the group exposed over 16 mo to diesel exhaust. No lung tumors were reported in controls. The equivocal response may have been caused by the relatively short exposure durations (20 mo) and small numbers of animals examined. In more recent studies, for example, Mauderly et al. (1987), most of the tumors were detected in rats exposed for more than 24 mo.

General Motors Research Laboratories sponsored chronic inhalation studies at the Southwest Research Institute using male Fischer 344 rats, 30 per group, exposed to DPM concentrations of 0.25, 0.75, or 1.5 mg/m^3 (Kaplan et al., 1983; White et al., 1983). The animals were exposed in 12.6 m^3 exposure chambers. Airflow was adjusted to provide 13 changes per hour. Temperature was maintained at 22 ± 2 °C. The exposure protocol was 20 hr/day, 7 days/week for 9 to 15 mo. Exposures were halted during normal working hours for servicing. Some animals were sacrificed following completion of exposure, while others were returned to clean air atmospheres for an additional 8 mo. Control animals received clean air. Exhaust was generated by 5.7-L Oldsmobile engines (four different engines used throughout the experiment) operated at a steady speed and load simulating a 40-mph driving speed of a full-size passenger car. Details of the exhaust-generating system and exposure atmosphere are presented in Appendix A.

Although five instances of bronchoalveolar carcinoma were observed in 90 rats exposed to diesel exhaust for 15 mo and held an additional 8 mo in clean air, compared with none among controls, statistical significance was not achieved in any of the exposure groups. These included one tumor in the 0.25 mg/m^3 group, three in the 0.75 mg/m^3 group, and one in the 1.5 mg/m^3 group. Rats kept in clean-air chambers for 23 mo did not exhibit any carcinomas. No tumors were observed in any of the 180 rats exposed to diesel exhaust for 9 or 15 mo without a recovery period, or in the respective controls for these groups. Equivocal results may again have been due to less-than-lifetime duration of the study as well as insufficient exposure concentrations. Although the increases in tumor incidences in the groups exposed for 15 mo and held an additional 8 mo in clean air were not statistically significant, relative to controls, they were slightly greater than the historic background incidence of 3.7% for this specific lesion in this strain of rat (Ward, 1983). The first definitive studies linking inhaled diesel exhaust to induction of lung cancer in rats were reported by researchers in Germany, Switzerland, Japan, and the United States in the mid-to-late 1980s. In a study conducted at the Fraunhofer Institute of Toxicology and Aerosol Research, female Wistar rats were exposed for 19 hr/day, 5 days/week to both filtered

and unfiltered (total) diesel exhaust at an average particulate matter concentration of 4.24 mg/m^3. Animals were exposed for a maximum of 2.5 years. The exposure system as described by Heinrich et al. (1986a) used a 40 kilowatt 1.6-L diesel engine operated continuously under the U.S. 72 FTP driving cycle. The engines used European Reference Fuel with a sulfur content of 0.36%. Filtered exhaust was obtained by passing engine exhaust through a Luwa FP-65 HT 610 particle filter heated to 80 °C and a secondary series of filters (Luwa FP-85, Luwa NS-30, and Drager CH 63302) at room temperature. The filtered and unfiltered exhausts were diluted 1:17 with filtered air and passed through respective 12 m^3 exposure chambers. Mass median aerodynamic diameter of DPM was 0.35 ± 0.10 µm (mean ± SD). The gas-phase components of the diesel exhaust atmospheres are presented in Appendix A.

The effects of exposure to either filtered or unfiltered exhaust were described by Heinrich et al. (1986b) and Stöber (1986). Exposure to unfiltered exhaust resulted in 8 bronchoalveolar adenomas and 9 squamous cell tumors in 15 of 95 female Wistar rats examined, for a 15.8% tumor incidence. Although statistical analysis was not provided, the increase appears to be highly significant. In addition to the bronchioalveolar adenomas and squamous cell tumors, there was a high incidence of bronchioalveolar hyperplasia (99%) and metaplasia of the bronchioalveolar epithelium (65%). No tumors were reported among rats exposed to filtered exhaust (n = 92) or clean air (n = 96).

Mohr et al. (1986) provided a more detailed description of the lung lesions and tumors identified by Heinrich et al. (1986a,b) and Stöber (1986). Substantial alveolar deposition of carbonaceous particles was noted for rats exposed to the unfiltered diesel exhaust. Squamous metaplasia was observed in 65.3% of the rats breathing unfiltered diesel exhaust, but not in the control rats. Of nine squamous cell tumors, one was characterized as a Grade I carcinoma (borderline atypia, few to moderate mitoses, and slight evidence of stromal invasion), and the remaining eight were classified as benign keratinizing cystic tumors.

Iwai et al. (1986) examined the long-term effects of diesel exhaust inhalation on female F344 rats. The exhaust was generated by a 2.4-L displacement truck engine. The exhaust was diluted 10:1 with clean air at 20 °C to 25 °C and 50% relative humidity. The engines were operated at 1,000 rpm with an 80% engine load. These operating conditions were found to produce exhaust with the highest particle concentration and lowest NO_2 and SO_2 content. For those chambers using filtered exhaust, proximally installed high-efficiency particulate air (HEPA) filters were used. Three groups of 24 rats each were exposed to unfiltered diesel exhaust, filtered diesel exhaust, or filtered room air for 8 hr/day, 7 days/week for 24 mo. Particle concentration was 4.9 mg/m^3 for unfiltered exhaust. Concentrations of gas-phase exhaust components were 30.9 ppm NO_x, 1.8 ppm NO_2, 13.1 ppm SO_2, and 7.0 ppm CO.

No lung tumors were found in the 2-year control (filtered room air) rats, although one adenoma was noted in a 30-mo control rat, providing a spontaneous tumor incidence of 4.5%. No lung tumors were observed in rats exposed to filtered diesel exhaust. Nineteen of the 24 exposed to unfiltered exhaust survived for 2 years. Of these, 14 were randomly selected for sacrifice at this time. Four of the rats developed lung tumors; two of these were malignant. Five rats of this 2-year exposure group were subsequently placed in clean room air for 3 to 6 mo and four eventually (time not specified) exhibited lung tumors (three malignancies). Thus, the lung tumor incidence for total tumors was 42.1% (8/19) and 26.3% (5/19) for malignant tumors in rats exposed to whole diesel exhaust. The tumor types identified were adenoma (3/19), adenocarcinoma (1/19), adenosquamous carcinoma (2/19), squamous carcinoma (1/19), and large-cell carcinoma (1/19). The lung tumor incidence in rats exposed to whole diesel exhaust was significantly greater than that of controls ($p \leq 0.01$). Tumor data are summarized in Table 7-4. Malignant splenic lymphomas were detected in 37.5% of the rats in the filtered exhaust group and in 25.0% of the rats in the unfiltered exhaust group; these values were significantly ($p \leq 0.05$) greater than the 8.2% incidence noted in the control rats. The study demonstrates production of lung cancer in rats following 2-year exposure to unfiltered diesel exhaust. In addition, splenic malignant lymphomas occurred during exposure to both filtered and unfiltered diesel exhaust. This is the only report to date of tumor induction at an extrarespiratory site by inhaled diesel exhaust in animals.

A chronic (up to 24 mo) inhalation exposure study was conducted by Takemoto et al. (1986), in which female Fischer 344 rats were exposed to diesel exhaust generated by a 269-cc YANMAR-40CE NSA engine operated at an idle state (1,600 rpm). Exposures were 4 hours/day, 4 days/week. The animals were exposed in a 376-L exposure chamber. Air flow was maintained at 120 L/min. Exhaust was diluted to produce a particle concentration of 2-4 mg/m$^{3.}$ Concentrations of the gas-phase components of the exhaust are presented in Appendix A. When not exposed the animals were maintained in an air-conditioned room at a temperature of 24 ± 2°C and a relative humidity of 55 ± 5% with 12 hr of light and darkness. Temperature and humidity in the exposure chambers was not noted. The particle concentration of the diesel exhaust in the exposure chamber was 2 to 4 mg/m^3. B[a]P and 1-nitropyrene concentrations were 0.85 and 93 µg/g of particles, respectively. No lung tumors were reported in the diesel-exposed animals. It was also noted that the diesel engine employed in this study was originally used as an electrical generator and that its operating characteristics (not specified) were different from those for a diesel-powered automobile. However, the investigators deemed it suitable for assessing the effects of diesel emissions.

Mauderly et al. (1987) provided data affirming the carcinogenicity of automotive diesel engine exhaust in F344/Crl rats following chronic inhalation exposure. Male and female rats were exposed to diesel engine exhaust at nominal DPM concentrations of 0.35 (n = 366), 3.5 (n = 367), or 7.1 (n = 364) mg/m^3 for 7 hr/day, 5 days/week for up to 30 mo. Sham-exposed (n = 365) controls breathed filtered room air. A total of 230, 223, 221, and 227 of these rats (sham-exposed, low-, medium-, and high-exposure groups, respectively) were examined for lung tumors. These numbers include those animals that died or were euthanized during exposure and those that were terminated following 30 mo of exposure. The exhaust was generated by 1980 model 5.7-L Oldsmobile V-8 engines operated through continuously repeating U.S. Federal Test Procedure (FTP) urban certification cycles. The engines were equipped with automatic transmissions connected to eddy-current dynamometers and flywheels simulating resistive and inertial loads of a midsize passenger car. The D-2 diesel control fuel (Phillips Chemical Co.) met U.S. EPA certification standards and contained approximately 30% aromatic hydrocarbons and 0.3% sulfur. Following passage through a standard automotive muffler and tail pipe, the exhaust was diluted 10:1 with filtered air in a dilution tunnel and serially diluted to the final concentrations. The primary dilution process was such that particle coagulation was retarded. Mokler et al. (1984) provided a detailed description of the exposure system. The gas-phase components of the diesel exhaust atmospheres are presented in Appendix A. No exposure-related changes in body weight or life span were noted for any of the exposed animals, nor were there any signs of overt toxicity. Collective lung tumor incidence was greater (z statistic, $p \leq 0.05$) in the high (7.1 mg/m^3) and medium (3.5 mg/m^3) exposure groups (12.8% and 3.6%, respectively) versus the control and low (0.35 mg/m^3) exposure groups (0.9% and 1.3%, respectively). In the high-dose group the incidences of tumor types reported were adenoma (0.4%), adenocarcinomas plus squamous cell carcinomas (7.5%), and squamous cysts (4.9%). In the medium-dose group adenomas were reported in 2.3% of animals, adenocarcinomas plus squamous cell carcinomas in 0.5%, and squamous cysts in 0.9%. In the low-exposure group adenocarcinomas plus squamous cell carcinomas were detected in 1.3% of the rats. Using the same statistical analysis of specific tumor types, adenocarcinoma plus squamous cell carcinoma and squamous cyst incidence was significantly greater in the high-exposure group, and the incidence of adenomas was significantly greater in the medium-exposure group. A significant ($p<0.001$) exposure-response relationship was obtained for tumor incidence relative to exposure concentration and lung burden of DPM. These data are summarized in Table 7-4. A logistic regression model estimating tumor prevalence as a function of time, dose (lung burden of DPM), and sex indicated a sharp increase in tumor prevalence for the high dose level at about 800 days after the commencement of exposure. A less pronounced, but definite, increase in prevalence with time was predicted for the medium-dose level. Significant effects were not detected at the

low concentration. DPM (mg per lung) of rats exposed to 0.35, 3.5, or 7.1 mg of DPM/m^3 for 24 mo were 0.6, 11.5, and 20.8, respectively, and affirmed the greater-than-predicted accumulation that was the result of decreased particle clearance following high-exposure conditions.

In summary, this study demonstrated the pulmonary carcinogenicity of high concentrations of whole, diluted diesel exhaust in rats following chronic inhalation exposure. In addition, increasing lung particle burden resulting from this high-level exposure and decreased clearance was demonstrated. A logistic regression model presented by Mauderly et al. (1987) indicated that both lung DPM burden and exposure concentration may be useful for expressing exposure-effect relationships.

A long-term inhalation study (Ishinishi et al., 1988a; Takaki et al., 1989) examined the effects of emissions from a light-duty (LD) and a heavy-duty (HD) diesel engine on male and female Fischer 344/Jcl rats. The LD engines were 1.8-L, 4-cylinder, swirl-chamber-type power plants, and the HD engines were 11-L, 6-cylinder, direct-injection-type power plants. The engines were connected to eddy-current dynamometers and operated at 1,200 rpm (LD engines) and 1,700 rpm (HD engines). Nippon Oil Co. JIS No. 1 or No. 2 diesel fuel was used. The 30-mo whole-body exposure protocol (16 h/day, 6 days/week) used DPM concentrations of 0, 0.5, 1, 1.8, or 3.7 mg/m^3 from HD engines and 0, 0.1, 0.4, 1.1, or 2.3 mg/m^3 from LD engines. An analysis of gas-phase components is presented in Appendix A. The animals inhaled the exhaust emissions from 1700 to 0900 h. Sixty-four male rats and 59 to 61 female rats from each exposure group were evaluated for carcinogenicity.

For the experiments using the LD series engines, the highest incidence of hyperplastic lesions plus tumors (72.6%) was seen in the highest exposure (2.3 mg/m^3) group. However, this high value was the result of the 70% incidence of hyperplastic lesions; the incidence of adenomas was only 0.8% and that of carcinomas 1.6%. Hyperplastic lesion incidence was considerably lower for the lower exposure groups (9.7%, 4.8%, 3.3%, and 3.3% for the 1.1, 0.4, and 0.1 mg/m^3 and control groups, respectively). The incidence of adenomas and carcinomas, combining males and females, was not significantly different among exposure groups (2.4%, 4.0%, 0.8%, 2.4%, and 3.3% for the 2.3, 1.1, 0.4, and 0.1 mg/m^3 groups and the controls, respectively).

For the experiments using the HD series engines, the total incidence of hyperplastic lesions, adenomas, and carcinomas was highest (26.6%) in the 3.7 mg/m^3 exposure group. The incidence of adenomas plus carcinomas for males and females combined equaled 6.5%, 3.3%, 0%, 0.8%, and 0.8% at 3.7, 1.8, 1, and 0.4 mg/m^3 and for controls, respectively. A statistically significant difference was reported between the 3.7 mg/m^3 and the control groups for the HD series engines. The carcinomas were identified as adenomas, adenosquamous carcinomas, and squamous cell carcinomas. Although the number of each was not reported, it was noted that the

majority were squamous cell carcinomas. A progressive dose-response relationship was not demonstrated. Tumor incidence data for this experiment are presented in Table 7-4.

The Ishinishi et al. (1988a) study also included recovery tests in which rats exposed to whole diesel exhaust (DPM concentration of 0.1 or 1.1 mg/m^3 for the LD engine and 0.5 or 1.8 mg/m^3 for the HD engine) for 12 mo were examined for lung tumors following 6-, 12-, or 18-mo recovery periods in clean air. The incidences of neoplastic lesions were low, and pulmonary DPM burden was lower than for animals continuously exposed to whole diesel exhaust and not provided a recovery period. The only carcinoma observed was in a rat examined 12 mo following exposure to exhaust (1.8 mg/m^3) from the HD engine.

Brightwell et al. (1986, 1989) studied the effects of diesel exhaust on male and female F344 rats. The diesel exhaust was generated by a 1.5-L Volkswagen engine that was computer-operated according to the U.S. 72 FTP driving cycle. The engine was replaced after 15 mo. The engine emissions were diluted by conditioned air delivered at 800 m^3/h to produce the high-exposure (6.6 mg/m^3) diesel exhaust atmosphere. Further dilutions of 1:3 and 1:9 produced the medium- (2.2 mg/m^3) and low- (0.7 mg/m^3) exposure atmospheres. The CO and NO_x concentrations (mean ± SD) were 32 ± 11 ppm and 8 ± 1 ppm in the high-exposure concentration chamber. The inhalation exposures were conducted overnight to provide five 16-h periods per week for 2 years; surviving animals were maintained for an additional 6 mo.

For males and females combined, a 1.2% (3/260), 0.7% (1/144), 9.7% (14/144), and 38.5% (55/143) incidence of primary lung tumors occurred in F344 rats following exposure to clean air or 0.7, 2.2, and 6.6 mg of DPM/m^3, respectively (Table 7-4). Diesel exhaust-induced tumor incidence in rats was dose-related and higher in females than in males (Table 7-4). These data included animals sacrificed at the interim periods (6, 12, 18, and 24 mo); therefore, the tumor incidence does not accurately reflect the effects of long-term exposure to the diesel exhaust atmospheres. When tumor incidence is expressed relative to the specific intervals, a lung tumor incidence of 96% (24/25), 76% (19/25) of which were malignant, was reported for female rats in the high-dose group exposed for 24 mo and held in clean air for the remainder of their lives. For male rats in the same group, the tumor incidence equaled 44% (12/27), of which 37% (10/27) were malignant. It was also noted that many of the animals exhibiting tumors had more than one tumor, often representing multiple histological types. The numbers and types of tumors identified in the rats exposed to diesel exhaust included adenomas (40), squamous cell carcinomas (35), adenocarcinomas (19), mixed adenoma/adenocarcinomas (9), and mesothelioma (1). It should be noted that exposure during darkness (when increased activity would result in greater respiratory exchange and greater inhaled dose) could account, in part, for the high response reported for the rats.

Lewis et al. (1989) also examined the effects of inhalation exposure of diesel exhaust and/or coal dust on tumorigenesis on F344 rats. Groups of 216 male and 72 female rats were exposed to clean air, whole diesel exhaust (2 mg soot/m^3), coal dust (2 mg/m^3 respirable concentration; 5 to 6 mg/m^3 total concentration), or diesel exhaust plus coal dust (1 mg/m^3 of each respirable concentration; 3.2 mg/m^3 total concentration) for 7 h/day, 5 days/week during daylight hours for up to 24 mo. Groups of 10 or more males were sacrificed at intermediate intervals (3, 6, and 12 mo). The diesel exhaust was produced by a 7.0-L, 4-cycle, water-cooled Caterpillar Model 3304 engine using No. 2 diesel fuel (<0.5% sulfur by mass). The exhaust was passed through a Wagner water scrubber, which lowered the exhaust temperature and quenched engine backfire. The animals were exposed in 100-cubic-foot chambers. Temperature was controlled at 22±2 °C and relative humidity at 50±10%. The exhaust was diluted 27-fold with chemically and biologically filtered clean air to achieve the desired particle concentration. An analysis of the exposure atmospheres is presented in Appendix A.

Histological examination was performed on 120 to 121 male and 71 to 72 female rats terminated after 24 mo of exposure. The exhaust exposure did not significantly affect the tumor incidence beyond what would be expected for aging F344 rats. There was no postexposure period, which may explain, in part, the lack of significant tumor induction. The particulate matter concentration was also less than the effective dose in several other studies.

In a more recent study reported by Heinrich et al. (1995), female Wistar rats were exposed to whole diesel exhaust (0.8, 2.5, or 7.0 mg/m^3) 18 h/day, 5 days/week for up to 24 mo, then held in clean air an additional 6 mo. The animals were exposed in either 6 or 12 m^3 exposure chambers. Temperature and relative humidity were maintined at 23-25 °C and 50%-70%, repectively. Diesel exhaust was generated by two 40-kw 1.6-L diesel engines (Volkswagen). One of them was operated according to the U.S. 72 cycle. The other was operated under constant load conditions. The first engine did not supply sufficient exhaust, which was filled by the second engine. Cumulative exposures for the rats in the various treatment groups were 61.7, 21.8, and 7.4 g/m^3 × h for the high, medium, and low whole-exhaust exposures. Significant increases in tumor incidences were observed in the high (22/100; p<0.001) and mid (11/200; p<0.01) exposure groups relative to clean-air controls (Table 7-4). Only one tumor (1/217), an adenocarcinoma, was observed in clean-air controls. Relative to clean-air controls, significantly increased incidences were observed in the high-exposure rats for benign squamous cell tumors (14/100; p<0.001), adenomas (4/100; p<0.01), and adenocarcinomas (5/100; p<0.05). Only the incidence of benign squamous cell tumors (7/200; p<0.01) was significantly increased in the mid-exposure group relative to the clean-air controls.

Particle lung burden and alveolar clearance also were determined in the Heinrich et al. (1995) study. Relative to clean air controls, alveolar clearance was significantly compromised by

exposure to mid and high diesel exhaust. For the high-diesel-exhaust group, 3-mo recovery time in clean air failed to reverse the compromised alveolar clearance.

In a study conducted at the Inhalation Toxicology Research Institute (Nikula et al., 1995) F344 rats (114-115 per sex per group) were exposed 16 hr/day, 5 days/week during daylight hours to diesel exhaust diluted to achieve particle concentrations of 2.5 or 6.5 mg/m^3 for up to 24 mo. Controls (118 males, 114 females) were exposed to clean air. Surviving rats were maintained an additional 6 weeks in clean air, at which time mortality reached 90%. Diesel exhaust was generated using two 1988 Model LH6 General Motors 6.2-L V-8 engines burning D-2 fuel that met EPA certification standards. Chamber air flow was sufficient to provide about 15 exchanges per hour. Relative humidity was 40% to 70% and temperature ranged from 23 to 25 °C.

Following low and high diesel exhaust exposure, the lung burdens were 36.7 and 80.7 mg, respectively, for females and 45.1 and 90.1 mg, respectively, for males. The percentages of susceptible rats (males and females combined) with malignant neoplasms were 0.9 (control), 3.3 (low diesel exhaust), and 12.3 (high diesel exhaust). The percentages of rats (males and females combined) with malignant or benign neoplasms were 1.4 (control), 6.2 (low diesel exhaust), and 17.9 (high diesel exhaust). All primary neoplasms were associated with the parenchyma rather than the conducting airways of the lungs. The first lung neoplasm was observed at 15 mo. Among 212 males and females examined in the high-dose group, adenomas were detected in 23 animals, adenocarcinomas in 22 animals, squamous cell carcinomas in 3 animals, and an adenosquamous carcinoma in 1 animal. For further details see Table 7-4. Analysis of the histopathologic data suggested a progressive process from alveolar epithelial hyperplasia to adenomas and adenocarcinomas.

Iwai et al. (1997) carried out a series of exposures to both filtered and whole exhaust using a light-duty (2,369 mL) diesel engine. The protocol for engine operation was not stated. Groups of female SPF F344 Fischer rats were exposed for 2 years for 8 hr/day, 7 days/week, 8 hr/day, 6 days/week, or 18 hr/day, 3 days/week to either filtered exhaust or exhaust diluted to a particle concentration of 9.4, 3.2, and 5.1 mg/m^3, respectively. Cumulative exposure (mg/m^3 × hrs of exposure) equaled 274.4, 153.6, and 258.1 mg/m^3. The animals were then held for an additional 6 mo in clean air. Lung tumors were reported in 5/121 (4%) of controls, 4/108 (4%) of those exposed to filtered exhaust, and 50/153 (35%) among those exposed to whole exhaust. Among rats exposed to whole diesel exhaust the following number of tumors were detected; 57 adenomas, 24 adenocarcinomas, 2 benign squamous cell tumors, 7 squamous cell carcinomas, and 3 adenosquamous carcinomas. The authors stated that benign squamous cell tumors probably corresponded to squamous cysts in another classification.

7.3.1.2. *Mouse Studies*

A series of inhalation studies using strain A mice was conducted by Orthoefer et al. (1981). Strain A mice are usually given a series of intraperitoneal injections with the test agent; they are then sacrificed at about 9 mo and examined for lung tumors. In the present series, inhalation exposure was substituted. Diesel exhaust was provided by one of two Nissan CN6-33 diesel engines having a displacement of 3244 cc and run on a Federal Short Cycle. Flow through the exposure chambers was sufficient to provide 15 air changes per hour. Temperature was maintained at 24 °C and relative humidity at 75%. In the first study, groups of 25 male Strong A strain (A/S) mice were exposed to irradiated diesel exhaust (to simulate chemical reactions induced by sunlight) or nonirradiated diesel exhaust (6 mg/m^3) for 20 h/day, 7 days/week. Additional groups of 40 Jackson A strain (S/J) mice (20 of each sex) were exposed similarly to either clean air or diesel exhaust, then held in clean air until sacrificed at 9 mo of age. No tumorigenic effects were detected at 9 mo of age. Further studies were conducted in which male A/S mice were exposed 8 hr/day, 7 days/week until sacrifice (approximately 300 at 9 mo of age and approximately 100 at 12 mo of age). With the exception of those treated with urethan, the number of tumors per mouse did not exceed historical control levels in any of the studies. Exposure to diesel exhaust, however, significantly inhibited the tumorigenic effects of the 5-mg urethan treatment. Results are listed in Table 7-4.

Kaplan et al. (1982) also reported the effects of diesel exposure in strain A mice. Groups of male strain A/J mice were exposed for 20 h/day, 7 days/week for 90 days and held until 9 mo of age. Experimental conditions are described in Appendix A. Briefly, the animals were exposed in inhalation chambers to diesel exhaust generated by a 5.7-L Oldsmobile engine operated continuously at 40 mph at DPM concentrations of 0, 0.25, 0.75, or 1.5 mg/m^3. Controls were exposed to clean air. Temperture was maintained at 22 ± 2 °C and relative humidity at 50 ± 10% within the chambers. Among 458 controls and 485 exposed animals, tumors were detected in 31.4% of those breathing clean air versus 34.2% of those exposed to diesel exhaust. The mean number of tumors per mouse also failed to show significant differences.

In a follow-up study, strain A mice were exposed to diesel exhaust for 8 mo (Kaplan et al., 1983; White et al., 1983). After exposure to the highest exhaust concentration (1.5 mg/m^3), the percentage of mice with pulmonary adenomas and the mean number of tumors per mouse were significantly less (p<0.05) than those for controls (25.0% vs. 33.5% and 0.30 ± 0.02 [S.E.] vs. 0.42 ± 0.03 [S.E.]) (Table 7-4).

Pepelko and Peirano (1983) summarized a series of studies on the health effects of diesel emissions in mice. Exhaust was provided by two Nissan CN 6-33, 6-cylinder, 3.24-L diesel engines coupled to a Chrysler A-272 automatic transmission and Eaton model 758-DG dynamometer. Details of the exposure atmosphere are presented in Appendix A. Sixty-day pilot

studies were conducted at a 1:14 dilution, providing DPM concentrations of 6 mg/$m^{3.}$ The engines were operated using the Modified California Cycle. These 20-hr/day, 7-days/week pilot studies using rats, cats, guinea pigs, and mice produced decreases in weight gain and food consumption. Therefore, at the beginning of the long-term studies, exposure time was reduced to 8 h/day, 7 days/week at an exhaust DPM concentration of 6 mg/m^3. During the final 12 mo of exposure, however, the DPM concentration was increased to 12 mg/m^3. For the chronic studies, the engines were operated using the Federal Short Cycle. Chamber temperature was maintained at 24 °C and relative humidity at 50%. Airflow was sufficient for 15 changes per hour.

Pepelko and Peirano (1983) described a two-generation study using Sencar mice exposed to diesel exhaust. Male and female parent-generation mice were exposed to diesel exhaust at a DPM concentration of 6 mg/m^3 prior to (from weaning to sexual maturity) and throughout mating. The dams continued exposure through gestation, birth, and weaning. Groups of offspring (130 males and 130 females) were exposed to either diesel exhaust or clean air. The exhaust exposure was increased to a DPM concentration of 12 mg/m^3 when the offspring were 12 weeks of age and was maintained until termination of the experiment when the mice were 15 mo old.

The incidence of pulmonary adenomas (16.3%) was significantly increased in the mice exposed to diesel exhaust compared with 6.3% in clean-air controls. The incidence in males and females combined was 10.2% in 205 animals examined compared with 5.1% in 205 clean-air controls. This difference was also significant. The incidence of carcinomas was not affected by exhaust exposure in either sex. These results provided the earliest evidence for cancer induction following inhalation exposure to diesel exhaust. The increase in the sensitivity of the study, allowing detection of tumors at 15 mo, may have been the result of exposure from conception. It is likely that Sencar mice are sensitive to induction of lung tumors since they are also sensitive to induction of skin tumors. These data are summarized in Table 7-4.

Takemoto et al. (1986) reported the effects of inhaled diesel exhaust (2 to 4 mg/m^3, 4 h/day, 4 days/week, for up to 28 mo) in ICR and C57BL mice exposed from birth. Details of the exposure conditions are presented in Section 7.3.2.1 and Appendix A. All numbers reported are for males and females combined. Four adenomas and 1 adenocarcinoma were detected in 34 diesel exhaust-exposed ICR mice autopsied at 13 to 18 mo, compared with 3 adenomas among 38 controls. Six adenomas and 3 adenocarcinomas were reported in 22 diesel-exposed ICR mice autopsied at 19 to 28 mo, compared with 3 adenomas and 1 adenocarcinoma in 22 controls. Four adenomas and 2 adenocarcinomas were detected in 79 C57BL mice autopsied at 13 to 18 mo, compared with none in 19 unexposed animals. Among males and females autopsied at 19 to 28 mo, 8 adenomas and 3 adenocarcinomas were detected in 71 exposed animals, compared with 1 adenoma among 32 controls. No significant increases in either adenoma or adenocarcinoma were

reported for either strain of exposed mice. However, the significance of the increase in the combined incidence of adenomas and carcinomas was not evaluated statistically. A statistical analysis by Pott and Heinrich (1990) indicated that the difference in combined benign and malignant tumors between whole diesel exhaust-exposed C57BL/6N mice and corresponding controls was significant at $p<.05$. See Table 7-4 for details of tumor incidence.

Heinrich et al. (1986b) and Stöber (1986), as part of a larger study, also evaluated the effects of diesel exhaust in mice. Details of the exposure conditions reported by Heinrich et al. (1986a) are given in Section 7.3.1.1 and Appendix A. Following lifetime (19 h/day, 5 days/week, for a maximum of 120 weeks) exposure to diesel exhaust diluted to achieve a particle concentration of 4.2 mg/m^3, 76 female NMRI mice exhibited a total lung tumor incidence of adenomas and adenocarcinomas combined of 32%. Tumor incidences reported for control mice (n = 84) equaled 11% for adenomas and adenocarcinomas combined. While the incidence of adenomas showed little change, adenocarcinomas increased significantly from 2.4% for controls to 17% for exhaust-exposed mice. In a follow up study, however, Heinrich et al. (1995) reported a lack of tumorigenic response in either female NMRI or C57BL/6N mice exposed 17 h/day, 5 days/week for 13.5 to 23 mo to whole diesel exhaust diluted to produce a particle concentration of 4.5 mg/m^3. These data are summarized in Table 7-4.

The lack of a carcinogenic response in mice was reported by Mauderly et al. (1996). In this study, groups of 540 to 600 CD-1 male and female mice were exposed to whole diesel exhaust (7.1, 3.5, or 0.35 mg DPM/m^3) for 7 hr/day, 5 days/week for up to 24 mo. Controls were exposed to filtered air. Diesel exhaust was provided by 5.7-L Oldsmobile V-8 engines operated continuously on the U.S. Federal Test Procedure urban certification cycle. The chambers were maintained at 25-28 °C, relative humidity at 40%-60%, and a flow rate sufficient for 15 air exchanges per hour. Animals were exposed during the light cycle, which ran from 6:00 AM to 6:00 PM. DPM accumulation in the lungs of exposed mice was assessed at 6, 12, and 18 mo of exposure and was shown to be progressive; DPM burdens were 0.2 ± 0.02, 3.7 ± 0.16, and 5.6 ± 0.39 mg for the low-, medium-, and high-exposure groups, respectively. The lung burdens in both the medium- and high-exposure groups exceeded that predicted by exposure concentration ratio for the low-exposure group. Contrary to what was observed in rats (Heinrich et al., 1986b; Stöber, 1986; Nikula et al., 1995; Mauderly et al., 1987), an exposure-related increase in primary lung neoplasms was not observed in the CD-1 mice, supporting the contention of a species difference in the pulmonary carcinogenic response to poorly soluble particles. The percentage incidence of mice (males and females combined) with one or more malignant or benign neoplasms was 13.4, 14.6, 9.7, and 7.5 for controls and low-, medium-, and high-exposure groups, respectively.

While earlier studies provided some evidence for tumorigenic responses in diesel-exposed mice, no increases were reported in the two most recent studies by Mauderly et al. (1996) and Heinrich et al. (1995), which utilized large group sizes and were well designed and conducted. Overall, the results in mice must therefore be considered to be equivocal.

7.3.1.3. *Hamster Studies*

Heinrich et al. (1982) examined the effects of diesel exhaust exposure on tumor frequency in female Syrian golden hamsters. Groups of 48 to 72 animals were exposed to clean air or whole diesel exhaust at a mean DPM concentration of 3.9 mg/m^3. Inhalation exposures were conducted 7 to 8 hr/day, 5 days/week for 2 years. The exhaust was produced by a 2.4-L Daimler-Benz engine operated under a constant load and a constant speed of 2,400 rpm. Flow rate was sufficient for about 20 exchanges per hour in the 250-L chambers. No lung tumors were reported in either exposure group.

In a subsequent study, Syrian hamsters were exposed 19 hr/day, 5 days/week for a lifetime to diesel exhaust diluted to a DPM concentration of 4.24 mg/m^3 (Heinrich et al., 1986b; Stöber, 1986). Details of the exposure conditions are reported in Appendix A. Ninety-six animals per group were exposed to clean air or exhaust. No lung tumors were seen in either the clean-air group or in the diesel exhaust-exposed group.

In a third study (Heinrich et al., 1989b), hamsters were exposed to exhaust from a Daimler-Benz 2.4-L engine operated at a constant load of about 15 kW and at a uniform speed of 2,000 rpm. The exhaust was diluted to an exhaust-clean air ratio of about 1:13, resulting in a mean particle concentration of 3.75 mg/m^3. Exposures were conducted in chambers maintained at 22 to 24 °C and 40% to 60% relative humidity for up to 18 mo. Surviving hamsters were maintained in clean air for up to an additional 6 mo. The animals were exposed 19 hr/day, 5 days/week beginning at noon each day, under a 12-hr light cycle starting at 7 AM. Forty animals per group were exposed to whole diesel exhaust or clean air. No lung tumors were detected in either the clean-air or diesel-exposed hamsters.

Brightwell et al. (1986, 1989) studied the effects of diesel exhaust on male and female Syrian golden hamsters. Groups of 52 males and 52 females, 6 to 8 weeks old, were exposed to diesel exhaust at DPM concentrations of 0.7, 2.2, or 6.6 mg/m^3. They were exposed 16 hr/day, 5 days/week for a total of 2 years and then sacrificed. Exposure conditions are described in Section 7.3.1.1 and in Appendix A. No statistically significant (*t* test) relationship between tumor incidence and exhaust exposure was reported.

In summary, diesel exhaust alone did not induce an increase in lung tumors in hamsters of either sex in several studies of chronic duration at high exposure concentrations.

7.3.1.4. *Monkey Studies*

Fifteen male cynomolgus monkeys were exposed to diesel exhaust (2 mg/m^3) for 7 hr/day, 5 days/week for 24 mo (Lewis et al., 1989). The same numbers of animals were also exposed to coal dust (2 mg/m^3 respirable concentration; 5 to 6 mg/m^3 total concentration), diesel exhaust plus coal dust (1 mg/m^3 respirable concentration for each component; 3.2 mg/m^3 total concentration), or filtered air. Details of exposure conditions were listed previously in the description of the Lewis et al. (1989) study with rats (Section 7.3.1.1) and are listed in Appendix A.

None of the monkeys exposed to diesel exhaust exhibited a significantly increased incidence of preneoplastic or neoplastic lesions. It should be noted, however, that the 24-mo timeframe employed in this study may not have allowed the manifestation of tumors in primates, because this duration is only a small fraction of the monkeys' expected lifespan. In fact, there have been no near-lifetime exposure studies in nonrodent species.

7.3.2. Inhalation Studies (Filtered Diesel Exhaust)

Several studies have been conducted in which animals were exposed to diesel exhaust filtered to remove PM. Since these studies also included groups exposed to whole exhaust, details can be found in Sections 7.3.1.1 for rats, 7.3.1.2 for mice, and 7.3.1.3 for hamsters, and in Appendix A. Heinrich et al. (1986b) and Stöber (1986) reported negative results for lung tumor induction in female Wistar rats exposed to filtered exhaust diluted to produce an unfiltered particle concentration of 4.24 mg/m^3. Negative results were also reported in female Fischer 344 rats exposed to filtered exhaust diluted to produce an unfiltered particle concentration of 4.9 mg/m^3 (Iwai et al., 1986), in Fischer 344 rats of either sex exposed to filtered exhaust diluted to produce an unfiltered particle concentration of 6.6 mg/m^3 (Brightwell et al., 1989), in female Wistar rats exposed to filtered exhaust diluted to produce an unfiltered particle concentration of 7.0 mg/m^3 (Heinrich et al., 1995), and in female Fischer 344 rats exposed to filtered exhaust diluted to produce unfiltered particle concentrations of 5.1, 3.2, or 9.4 mg/m^3 (Iwai et al., 1997). In the Iwai et al. (1986) study, splenic lymphomas were detected in 37.5% of the exposed rats compared with 8.2% in controls.

In the study reported by Heinrich at al. (1986a) and Stober (1986), primary lung tumors were seen in 29/93 NMRI mice (males and females combined) exposed to filtered exhaust, compared with 11/84 in clean-air controls, a statistically significant increase. In a repeat study by Heinrich et al. (1995), however, significant lung tumor increases were not detected in either female NMRI or C57BL/6N mice exposed to filtered exhaust diluted to produce an unfiltered particle concentration of 4.5 mg/m^3.

Filtered exhaust also failed to induce lung tumor induction in Syrian Golden hamsters (Heinrich et al., 1986a; Brightwell et al., 1989).

Although lung tumor increases were reported in one study and lymphomas in another, these results could not be confirmed in subsequent investigations. It is therefore concluded that little direct evidence exists for carcinogenicity of the vapor phase of diesel exhaust in laboratory animals at concentrations tested.

7.3.3. Inhalation Studies (Diesel Exhaust Plus Co-Carcinogens)

Details of the studies reported here have been described earlier and in Table 7-4. Tumor initiation with urethan (1 mg/kg body weight i.p. at the start of exposure) or promotion with butylated hydroxytolulene (300 mg/kg body weight i.p. week 1, 83 mg/kg week 2, and 150 mg/kg for weeks 3-52) did not influence tumorigenic responses in Sencar mice of both sexes exposed to concentrations of diesel exhaust up to 12 mg/m^3 (Pepelko and Peirano, 1983).

Heinrich et al. (1986b) exposed Syrian hamsters of both sexes to diesel exhaust diluted to a particle concentration of 4 mg/m^3. See Section 7.3.1.1 for details of the exposure conditions. At the start of exposure the hamsters received either one dose of 4.5 mg diethylnitrosamine (DEN) subcutaneously per kg body weight or 20 weekly intratracheal instillations of 250 µ*g* BaP. Female NMRI mice received weekly intratracheal instillations of 50 or 100 µ*g*BaP for 10 or 20 weeks, respectively, or 50 µ*g* dibenz[ah]anthracene (DBA) for 10 weeks. Additional groups of 96 newborn mice received one s.c. injection of 5 or 10 µ*g* DBA between 24 and 48 hr after birth. Female Wistar rats received weekly subcutaneous injections of dipentylnitrosamine (DPN) at doses of 500 and 250 mg/kg body weight, respectively, during the first 25 weeks of exhaust inhalation exposure. Neither DEN, DBA, or DPN treatment enhanced any tumorigenic responses to diesel exhaust. Although response to BaP did not differ from that of BaP alone in hamsters, results were inconsistent in mice. Although 20 BaP instillations induced a 71% tumor incidence in mice, concomitant diesel exposure resulted in only a 41% incidence. However, neither 10 BaP instillations nor DBA instillations induced significant effects.

Takemoto et al. (1986) exposed Fischer 344 rats for 2 years to diesel exhaust at particle concentrations of 2 to 4 mg/m^3. One month after start of inhalation exposure one group of rats received di-isopropyl-nitrosamine (DIPN) administered i.p. at 1 mg/kg weekly for 3 weeks. Among injected animals autopsied at 18 to 24 mo, 10 adenomas and 4 adenocarcinomas were reported in 21 animals exposed to clean air, compared with 12 adenomas and 7 adenocarcinomas in 18 diesel-exposed rats. According to the authors, the incidence of adenocarcinomas was not significantly increased by exposure to diesel exhaust.

Brightwell et al. (1989) investigated the concomitant effects of diesel exhaust and DEN in Syrian hamsters exposed to diesel exhaust diluted to produce particle concentrations of 0.7, 2.2, or 6.6 mg/m^3 for 2 years. The animals received a single dose of 4.5 mg DEN s.c. 3 days prior to start of inhalation exposure. DEN did not affect the lack of responsiveness to diesel exhaust

alone. Heinrich et al. (1989b) also exposed Syrian hamsters of both sexes to diesel exhaust diluted to a particle concentration of 3.75 mg/m^3 for up to 18 mo. After 2 weeks of exposure, groups were treated with either 3 or 6 mg DEN/kg body weight, respectively. Again, DEN did not significantly influence the lack of tumorigenic responses to diesel exhaust.

Heinrich et al. (1989a) investigated the effects of DPN in female Wistar rats exposed to diesel exhaust diluted to achieve a particle concentration of 4.24 mg/m^3 for 2-2.5 years. DPN at doses of 250 and 500 mg/kg body weight was injected subcutaneously once a week for the first 25 weeks of exposure. The tumorigenic responses to DPN were not affected by exposure to diesel exhaust. For details of exposure conditions of the hamster studies see Section 7.3.1.3.

Heinrich et al. (1986a) and Mohr et al. (1986) compared the effects of exposure to particles having only a minimal carbon core but a much greater concentration of PAHs than DPM does. The desired exposure conditions were achieved by mixing coal oven flue gas with pyrolyzed pitch. The concentration of B[a]P and other PAHs per milligram of DPM was about three orders of magnitude greater than that of diesel exhaust. Female rats were exposed to the flue gas-pyrolyzed pitch for 16 hr/day, 5 days/week at particle concentrations of 3 to 7 mg/m^3 for 22 mo, then held in clean air for up to an additional 12 mo. Among 116 animals exposed, 22 tumors were reported in 21 animals, for an incidence of 18.1%. One was a bronchioloalveolar adenoma, one was a bronchioloalveolar carcinoma, and 20 were squamous cell tumors. Among the latter, 16 were classified as benign keratinizing cystic tumors and 4 were classified as carcinomas. No tumors were reported in 115 controls. The tumor incidence in this study was comparable to that reported previously for the diesel exhaust-exposed animals.

In analyzing the studies of Heinrich et al. (1986a,b), Heinrich (1990), Mohr et al. (1986), and Stöber (1986), it must be noted that the incidence of lung tumors occurring following exposure to whole diesel exhaust, coal oven flue gas, or carbon black (15.8%, 18.1%, and 8% to 17%, respectively) was very similar. This occurred despite the fact that the PAH content of the PAH-enriched pyrolyzed pitch was more than three orders of magnitude greater than that of diesel exhaust; carbon black, on the other hand, had only traces of PAHs. Based on these findings, particle-associated effects appear to be the primary cause of diesel-exhaust-induced lung cancer in rats exposed at high concentrations. This issue is discussed further in Chapter 7.

7.3.4. Lung Implantation or Intratracheal Instillation Studies

7.3.4.1. *Rat Studies*

Grimmer et al. (1987), using female Osborne Mendel rats (35 per treatment group), provided evidence that PAHs in diesel exhaust that consist of four or more rings have carcinogenic potential. Condensate was obtained from the whole exhaust of a 3.0-L passenger-car diesel engine connected to a dynamometer operated under simulated city traffic driving

conditions. This condensate was separated by liquid-liquid distribution into hydrophilic and hydrophobic fractions representing 25% and 75% of the total condensate, respectively. The hydrophilic, hydrophobic, or reconstituted hydrophobic fractions were surgically implanted into the lungs of the rats. Untreated controls, vehicle (beeswax/trioctanoin) controls, and positive (B[a]P) controls were also included in the protocol (Table 7-5). Fraction Ilb (made up of PAHs with four to seven rings), which accounted for only 0.8% of the total weight of DPM condensate, produced the highest incidence of carcinomas following implantation into rat lungs. A carcinoma incidence of 17.1% was observed following implantation of 0.21 mg IIb/rat, whereas the nitro-PAH fraction (IId) at 0.18 mg/rat accounted for only a 2.8% carcinoma incidence. Hydrophilic fractions of the DPM extracts, vehicle (beeswax/trioctanoin) controls, and untreated controls failed to exhibit carcinoma formation. Administration of all hydrophobic fractions (IIa-d) produced a carcinoma incidence (20%) similar to the summed incidence of fraction IIb (17.1%) and IId (2.8%). The B[a]P positive controls (0.03, 0.1, 0.3 mg/rat) yielded a carcinoma incidence of 8.6%, 31.4%, and 77.1%, respectively. The study showed that the tumorigenic agents were primarily four- to seven-ring PAHs and, to a lesser extent, nitroaromatics. However, these studies demonstrated that simultaneous administration of various PAH compounds resulted in a varying of the tumorigenic effect, thereby implying that the tumorigenic potency of PAH mixtures may not depend on any one individual PAH. This study did not provide any information regarding the bioavailability of the particle-associated PAHs that might be responsible for carcinogenicity.

Kawabata et al. (1986) compared the effects of activated carbon and diesel exhaust on lung tumor formation. One group of 59 F344 rats was intratracheally instilled with DPM (1 mg/week for 10 weeks). A second group of 31 rats was instilled with activated carbon using the same dosing regime. Twenty-seven rats received only the solvent (buffered saline with 0.05% Tween 80), and 53 rats were uninjected. Rats dying after 18 mo were autopsied. All animals surviving 30 mo or more postinstillation were sacrificed and evaluated for histopathology. Among 42 animals exposed to DPM surviving 18 mo or more, tumors were reported in 31, including 20 malignancies. In the subgroup surviving for 30 mo, tumors were detected in 19 of 20 animals, including 10 malignancies. Among the rats exposed to activated carbon, the incidence of lung tumors equaled 11 of 23 autopsied, with 7 cases of malignancy. Data for those dying between 18 and 30 mo and those sacrificed at 30 mo were not reported separately.

Table 7-5. Tumor incidence and survival time of rats treated by surgical lung implantation with fractions from diesel exhaust condensate (35 rats/group)

Material portion by weight (%)	Dose (mg)	Median survival time in weeks (range)	Number of carcinomas[a]	Number of adenomas[b]	Carcinoma incidence (%)
Hydrophilic fraction (I) (25)	6.70	97 (24-139)	0	1	0
Hydrophobic fraction (II) (75)	20.00	99 (50-139)	5	0	14.2
Nonaromatics +					
PAC[c] 2 + 3 rings (IIa) (72)	19.22	103 (25-140)	0	1	0
PAH[d] 4 to 7 rings (IIb) (0.8)	0.21	102 (50-140)	6	0	17.1
Polar PAC (IIc) (1.1)	0.29	97 (44-138)	0	0	0
Nitro-PAH (IId) (0.7)	0.19	106 (32-135)	1	0	2.8
Reconstituted hydrophobics (Ia, b, c, d) (74.5)	19.91	93 (46-136)	7	1	20.0
Control, unrelated		110 (23-138)	0	0	0
Control (beeswax/trioctanoin)		103 (51-136)	0	1	0
Benzo[*a*]pyrene	0.3	69 (41-135)	27	0	77.1
	0.1	98 (22-134)	11	0	31.4
	0.03	97 (32-135)	3	0	8.6

[a]Squamous cell carcinoma.
[b]Bronchiolar/alveolar adenoma.
[c]PAC = polycyclic aromatic compounds.
[d]PAH = polycyclic aromatic hydrocarbons.

Source: Adapted from Grimmer et al., 1987.

Statistical analysis indicated that activated carbon induced a significant increase in lung tumor incidence compared with no tumors in 50 uninjected controls and 1 tumor in 23 solvent-injected controls. The tumor incidence was significantly greater in the DPM-instilled group and was significantly greater than the increase in the carbon-instilled group.

A study reported by Rittinghausen et al. (1997) suggested that organic constituents of diesel particles play a role in the induction of lung tumors in rats. An incidence of 16.7% pulmonary cystic keratinizing squamous cell lesions was noted in rats intratracheally instilled with 15 mg whole diesel exhaust particles, compared with 2.1% in rats instilled with 15 mg particles extracted to remove all organic constituents, and none among controls. Instillation of 30 mg of extracted particles induced a 14.6% incidence of squamous lesions, indicating the greater effectiveness of particles alone as lung particle overload increased.

Iwai et al. (1997) instilled 2, 4, 8, and 10 mg of whole diesel particles over a 2 to 10 week period into female F/344 rats, 50 or more per group. Tumors were reported in 6%, 20%, 43%, and 74% of the rats, with incidence of malignant tumors equal to 2%, 13%, 34%, and 48%, respectively. In a second experiment comparing whole with extracted diesel particles, tumor incidence equaled 1/48 (2%) in uninjected controls, 3/55 (5%) in solvent controls, 12/56 (21%) in extracted diesel particles, and 13/106 (12%) in animals injected with unextracted particles. Although the extracted particles appeared to be more potent, when converted to a lung burden basis (mg/100 mg dry lung) the incidence was only 14% among those exposed to extracted exhaust compared with 31% in those exposed to whole particles.

Dasenbrock et al. (1996) conducted a study to determine the relative importance of the organic constituents of diesel particles and particle surface area in the induction of lung cancer in rats. Fifty-two female Wistar rats were intratracheally instilled with 16-17 doses of DPM, extracted DPM, printex carbon black (PR), lampblack (LB), benzo[*a*]pyrene (B*a*P), DPM + B*a*P, or PR + B*a*P. The animals were held for a lifetime or sacrificed when moribund. The lungs were necropsied and examined for tumors. Diesel particles were collected from a Volkswagen 1.6-L engine operating on a US FTP-72 driving cycle. The mass median aerodynamic diameter (MMAD) of the diesel particles was 0.25 µm and the specific surface area was 12 m^2/gm. Following extraction with toluene, specific surface area increased to 138 m^2/gm. The MMAD for extracted PR was equal to 14 nm, while the specific surface area equaled 271 m^2/gm. The MMAD for extracted lampblack was equal to 95 nm, with a specific surface area equal to 20 m^2/gm. The B*a*P content of the treated particles was 11.3 mg per gm diesel particles and 29.5 mg B*a*P per gm PR. Significant increases in lung tumors were detected in rats instilled with 15 mg unextracted DPM and 30 mg extracted DPM, but not 15 mg extracted DPM. Printex CB was

more potent than lampblack CB for induction of lung tumors, while B*a*P was effective only at high doses. Total dose and tumor responses are shown in Table 7-6.

A number of conclusions can be drawn from these results. First of all, particles devoid of organics are capable of inducing lung tumor formation, as indicated by positive results in the groups treated with high-dose extracted diesel particles and printex. Nevertheless, toluene extraction of organics from diesel particles results in a decrease in potency, indicating that the organic fraction does play a role in cancer induction. A relationship between cancer potency and particle surface area was also suggested by the finding that printex with a large specific surface area was more potent than either extracted DPM or lampblack, which have smaller specific areas. Finally, while very large doses of B*a*P are very effective in the induction of lung tumors, smaller doses adsorbed to particle surfaces had little detectable effect, suggesting that other organic components of diesel exhaust may be of greater importance in the induction of lung tumors at low doses pf B*a*P (0.2-0.4 mg).

7.3.4.2. *Syrian Hamster Studies*

Kunitake et al. (1986) and Ishinishi et al. (1988b) conducted a study in which total doses of 1.5, 7.5, or 15 mg of a dichloromethane extract of DPM were instilled intratracheally over 15 weeks into male Syrian hamsters that were then held for their lifetimes. The tumor incidences of 2.3% (1/44), 0% (0/56), and 1.7% (1/59) for the high-, medium-, and low-dose groups, respectively, did not differ significantly from the 1.7% (1/56) reported for controls. Addition of 7.5 mg of B[a]P to a DPM extract dose of 1.5 mg resulted in a total tumor incidence of 91.2% and malignant tumor incidence of 88%. B[a]P (7.5 mg over 15 weeks) alone produced a tumor incidence rate of 88.2% (85% of these being malignant), which was not significantly different from the DPM extract + B[a]P group. Intratracheal administration of 0.03 µg B[a]P, the equivalent content in 15 mg of DPM extract, failed to cause a significant increase in tumors in rats. This study demonstrated a lack of detectable interaction between DPM extract and B[a]P, the failure of DPM extract to induce carcinogenesis, and the propensity for respiratory tract carcinogenesis following intratracheal instillation of high doses of B[a]P. For studies using the DPM extract, some concern must be registered regarding the known differences in chemical composition between DPM extract and DPM. As with all intratracheal instillation protocols, DPM extract lacks the complement of volatile chemicals found in whole diesel exhaust.

The effects on hamsters of intratracheally instilled DPM suspension, DPM with Fe_2O_3, or DPM extract with Fe_2O_3 as the carrier were studied by Shefner et al. (1982). The DPM component in each of the treatments was administered at concentrations of 1.25, 2.5, or 5.0

Table 7-6. Tumor incidences in rats following intratracheal instillation of diesel exhaust particles (DPM), extracted DPM, carbon black (CB), benzo[*a*]pyrene (B*a*P), or particles plus B*a*P

Experimental group	Number of animals	Total dose	Animals with tumors (percent)	Statistical significance[a]
Control	47	4.5 mL	0 (0)	-
DPM (original)	48	15 mg	8 (17)	< 0.01
DPM (extracted)	48	30 mg	10 (21)	< 0.001
DPM (extracted)	48	15 mg	2 (4)	NS
CB (printex)	48	15 mg	10 (21)	< 0.001
CB (lampblack)	48	14 mg	4 (8)	NS
B*a*P	47	30 mg	43 (90)	< 0.001
B*a*P	48	15 mg	12 (25)	< 0.001
DEP + B*a*P	48	15 mg + 170 µg B*a*P	4 (8)	NS
CB (printex) + B*a*P	48	15 mg + 443 µg B*a*P	13 (27)	< 0.001

[a]Fischer's exact test.

Source: Dasenbrock et al., 1996.

mg/week for 15 weeks to groups of 50 male Syrian golden hamsters. The total volume instilled was 3.0 mL (0.2 mL/week for 15 weeks). The DPM and dichloromethane extracts were suspended in physiological saline with gelatin (0.5% w/v), gum arabic (0.5% w/v), and propylene glycol (10% by volume). The Fe_2O_3 concentration, when used, was 1.25 mg/0.2 mL of suspension. Controls received vehicle and, where appropriate, carrier particles (Fe_2O_3) without the DPM component. Two replicates of the experiments were performed. Adenomatous hyperplasia was reported to be most severe in those animals treated with DPM or DPM plus Fe_2O_3 particles and least severe in those animals receiving DPM plus Fe_2O_3. Of the two lung adenomas detected microscopically, one was in an animal treated with a high dose of DPM and the other was in an animal receiving a high dose of DPM extract. Although lung damage was increased by instillation of DPM, there was no evidence of tumorigenicity.

7.3.4.3. *Mouse Studies*

Ichinose et al. (1997a) intratracheally instilled 36 four-week-old male ICR mice per group weekly for 10 weeks with sterile saline or 0.05, 0.1, or 0.2 mg DPM. Particles were collected from a 2.74-L four-cylinder Isuzu engine run at a steady speed of 1,500 rpm under a load of 10 torque (kg/m). Twenty-four hours after the last instillation, six animals per group were sacrificed for measurement of lung 8-hydroxydeoxyguanosine (8-OHdG). The remaining animals were sacrificed after 12 mo for histopathological analysis. Lung tumor incidence varied from 4/30 (13.3%) for controls to 9/30 (30%), 9/29 (31%), and 7/29 (24.1%) for mice instilled with 0.05, 0.1, and 0.2 mg/week, respectively. The increase in animals with lung tumors compared with controls was statistically significant for the 0.1 mg dose group, the only group analyzed statistically. Increases in 8-OHdG, an indicator of oxidative DNA damage, correlated well with the increase in tumor incidence in the 0.05 mg dose group, although less so with the other two. The correlation coefficients r = 0.916, 0.765, and 0.677 for the 0.05, 0.10, and 0.20 mg DPM groups, respectively.

In a similar study, 33 four-week-old male ICR mice per group were intratracheally instilled weekly for 10 weeks with sterile saline, 0.1 mg DPM, or 0.1 mg DPM from which the organic constituents were extracted with hexane (Ichinose et al., 1997b). Exhaust was collected from a 2.74-L four-cylinder Izuzu engine run at a steady speed of 2,000 rpm under a load of 6 torque (kg/m). Twenty-four hours after the last instillation, six animals per group were sacrificed for measurement of 8-OHdG. Surviving animals were sacrificed after 12 mo. The incidence of lung tumors increased from 3/27 (11.1%) among controls to 7/27 (25.9%) among those instilled with extracted diesel particles and 9/26 (34.6%) among those instilled with unextracted particles. The increase in number of tumor-bearing animals was statistically significant compared with controls (p<0.05) for the group treated with unextracted particles. The increase in 8-OHdG was highly correlated with lung tumor incidence, r = 0.99.

7.3.5. Subcutaneous and Intraperitoneal Injection Studies

7.3.5.1. *Mouse Studies*

In addition to inhalation studies, Orthoefer et al. (1981) also tested the effects of i.p. injections of DPM on male (A/S) strain mice. Three groups of 30 mice were injected with 0.1 mL of a suspension (particles in distilled water) containing 47, 117, or 235 µg of DPM collected from Fluoropore filters in the inhalation exposure chambers. The exposure system and exposure atmosphere are described in Appendix A. Vehicle controls received injections of particle suspension made up of particulate matter from control exposure filters, positive controls received 20 mg of urethan, and negative controls received no injections. Injections were made three times weekly for 8 weeks, resulting in a total DPM dose of 1.1, 2.8, and 5.6 mg for the low-, medium-,

and high-dose groups and 20 mg of urethan for the positive control group. These animals were sacrificed after 26 weeks and examined for lung tumors. For the low-, medium-, and high-dose DPM groups, the tumor incidence was 2/30, 10/30, and 8/30, respectively. The incidence among urethan-treated animals (positive controls) was 100% (29/29), with multiple tumors per animal. The tumor incidence for the DPM-treated animals did not differ significantly from that of vehicle controls (8/30) or negative controls (7/28). The number of tumors per mouse was also unaffected by treatment.

In further studies conducted by Orthoefer et al. (1981), an attempt was made to compare the potency of DPM with that of other environmental pollutants. Male and female Strain A mice were injected i.p. three times weekly for 8 weeks with DPM, DPM extracts, or various environmental mixtures of known carcinogenicity, including cigarette smoke condensate, coke oven emissions, and roofing tar emissions. Injection of urethan or dimethylsulfoxide (DMSO) served as positive or vehicle controls, respectively. In addition to DPM from the Nissan diesel previously described, an eight-cylinder Oldsmobile engine operated at the equivalent of 40 mph was also used to compare emission effects from different makes and models of diesel engine. The mice were sacrificed at 9 mo of age and their lungs examined for histopathological changes. The only significant findings, other than for positive controls, were small increases in numbers of lung adenomas per mouse in male mice injected with Nissan DPM and in female mice injected with coke oven extract. Furthermore, the increase in the extract-treated mice was significant only in comparison with uninjected controls (not injected ones) and did not occur when the experiment was repeated. Despite the use of a strain of mouse known to be sensitive to tumor induction, the overall findings of this study were negative. The authors provided several possible explanations for these findings, the most likely of which were (1) the carcinogens that were present were very weak, or (2) the concentrations of the active components reaching the lungs were insufficient to produce positive results.

Kunitake et al. (1986) conducted studies using DPM extract obtained from a 1983 HD MMC—6D22P 11-L V-6 engine. Five s.c. injections of DPM extract (500 mg/kg per injection) resulted in a significant ($p<0.01$) increase in subcutaneous tumors for female C57BL mice (5/22 [22.7%] vs. 0/38 among controls). Five s.c. doses of DPM extract of 10, 25, 30, 100, or 200 mg/kg failed to produce a significant increase in tumor incidence. One of 12 female ICR mice (8.3%) and 4 of 12 male ICR mice (33.3%) developed malignant lymphomas following neonatal s.c. administration of 10 mg of DPM extract per mouse. The increase in malignant lymphoma incidence for the male mice was statistically significant at $p<0.05$ compared with an incidence of 2/14 (14.3%) among controls. Treatment of either sex with 2.5 or 5 mg of DPM extract per mouse did not result in statistically significant increases in tumor incidence.

Additional studies using DPM extract from LD (1.8-L, 4-cylinder) as well as HD engines with female ICR and nude mice (BALB/c/cA/JCL-nu) were also reported (Kunitake et al., 1988). Groups of 30 ICR and nude mice each were given a single s.c. injection of 10 mg HD extract, 10 mg HD + 50 µg 12-O-tetradecanoylphorbol 13-acetate (TPA), 10 mg LD extract + 50 µg TPA, or 50 µg TPA. No malignant tumors or papillomas were observed. One papillomatous lesion was observed in an ICR mouse receiving LD extract + TPA, and acanthosis was observed in one nude mouse receiving only TPA.

In what appears to be an extension of the Kunitake et al. (1986) s.c. injection studies, Takemoto et al. (1988) presented additional data for subcutaneously administered DPM extract from HD and LD diesel engines. In this report, the extracts were administered to 5-week-old and neonatal (<24 hr old) C57BL mice of both sexes. DPM extract from HD or LD engines was administered weekly to the 5-week-old mice for 5 weeks at doses of 10, 25, 50, 100, 200, or 500 mg/kg, with group sizes ranging from 15 to 54 animals. After 20 weeks, comparison with a control group indicated a significant increase in the incidence of subcutaneous tumors for the 500 mg/kg HD group (5 of 22 mice [22.7%], $p<0.01$), the 100 mg/kg LD group (6 of 32 [18.8%], $p<0.01$), and the 500 mg/kg LD group (7 of 32 [21.9%], $p<0.01$) in the adult mouse experiments. The tumors were characterized as malignant fibrous histiocytomas. No tumors were observed in other organs. The neonates were given single doses of 2.5, 5, or 10 mg DPM extract subcutaneously within 24 hr of birth. There was a significantly higher incidence of malignant lymphomas in males receiving 10 mg of HD extract and of lung tumors for males given 2.5 mg HD extract and for males given 5 mg and females given 10 mg LD extract. A dose-related trend that was not significant was observed for the incidences of liver tumors for both the HD extract- and LD extract-treated neonatal mice. The incidence of mammary tumors in female mice and multiple-organ tumors in male mice was also greater for some extract-treated mice, but was not dose related. The report concluded that LD DPM extract showed greater carcinogenicity than did HD DPM extract.

7.3.6. Dermal Studies

7.3.6.1. *Mouse Studies*

In one of the earliest studies of diesel emissions, the effects of dermal application of extract from DPM were examined by Kotin et al. (1955). Acetone extracts were prepared from the DPM of a diesel engine (type and size not provided) operated at warmup mode and under load. These extracts were applied dermally three times weekly to male and female C57BL and strain A mice. Results of these experiments are summarized in Table 7-7. In the initial

Table 7-7. Tumorigenic effects of dermal application of acetone extracts of diesel particulate matter (DPM)

Number of animals	Strain/sex	Sample material	Time to first tumor (mo)	Survivors at time of first tumor	Total tumors	Duration of experiment (mo)
52	C57BL/40 F C57BL/12 M	Extract of DPM obtained during warmup	13	33	2	22
50	Strain A/M	Extract of DPM obtained during full load	15	8	4	23
25	Strain A/F	Extract of DPM obtained during full load	13	20	17	17

Source: Kotin et al. (1955).

experiments using 52 (12 male, 40 female) C57BL mice treated with DPM extract from an engine operated in warmup mode, two papillomas were detected after 13 mo. Four tumors were detected 16 mo after the start of treatment in 8 surviving of 50 exposed male strain A mice treated with DPM extract from an engine operated under full load. Among female strain A mice treated with DPM extract from an engine operated under full load, 17 tumors were detected in 20 of 25 mice surviving longer than 13 mo. This provided a significantly increased tumor incidence of 85%. Carcinomas as well as papillomas were seen, but the numbers were not reported.

Depass et al. (1982) examined the potential of DPM and dichloromethane extracts of DPM to act as complete carcinogens, carcinogen initiators, or carcinogen promoters. In skin-painting studies, the DPM was obtained from an Oldsmobile 5.7-L diesel engine operated under constant load at 65 km/h. The DPM was collected at a temperature of 100 °C. Groups of 40 C3H/HeJ mice were used because of their low spontaneous tumor incidence. For the complete carcinogenesis experiments, DPM was applied as a 5% or 10% suspension in acetone. Dichloromethane extract was applied as 5%, 10%, 25%, or 50% suspensions. Negative controls received acetone, and positive controls received 0.2% B[a]P. For tumor-promotion experiments, a single application of 1.5% B[a]P was followed by repeated applications of 10% DPM suspension, 50% DPM extract, acetone only (vehicle control), 0.0001% phorbol 12-myristate 13-acetate (PMA) as a positive promoter control, or no treatment (negative control). For the tumor-initiation studies, a single initiating dose of 10% diesel particle suspension, 50% diesel particle extract, acetone, or PMA was followed by repeated applications of 0.0001% PMA. Following 8 mo of treatment, the PMA dose in the initiation and promotion studies was increased to 0.01%. Animals were treated three times per week in the complete carcinogenesis and initiation experiments and five times per week in promotion experiments. All test compounds were applied to a shaved area on the back of the mouse.

In the complete carcinogenesis experiments, one mouse receiving the high-dose (50%) suspension of extract developed a squamous cell carcinoma after 714 days of treatment. Tumor incidence in the B[a]P group was 100%, and no tumors were observed in any of the other groups. For the promotion studies, squamous cell carcinomas with pulmonary metastases were identified in one mouse of the 50% DPM extract group and in one in the 25% extract group. Another mouse in the 25% extract group developed a grossly diagnosed papilloma. Nineteen positive control mice had tumors (11 papillomas, 8 carcinomas). No tumors were observed for any of the other treatment groups. For the initiation studies, three tumors (two papillomas and one carcinoma) were identified in the group receiving DPM suspension and three tumors (two papillomas and one fibrosarcoma) were found in the DPM extract group. These findings were reported to be statistically insignificant using the Breslow and Mantel-Cox tests.

Although these findings were not consistent with those of Kotin et al. (1955), the occurrence of a single carcinoma in a strain known to have an extremely low spontaneous tumor incidence may be of importance. Furthermore, a comparison between studies employing different strains of mice with varying spontaneous tumor incidences may result in erroneous assumptions.

Nesnow et al. (1982) studied the formation of dermal papillomas and carcinomas following dermal application of dichloromethane extracts from coke oven emissions, roofing tar, DPM, and gasoline engine exhaust. DPM from five different engines, including a preproduction Nissan 220C, a 5.7-L Oldsmobile, a prototype Volkswagen Turbo Rabbit, a Mercedes 300D, and a HD Caterpillar 3304, was used for various phases of the study. Male and female Sencar mice (40 per group) were used for tumor initiation, tumor promotion, and complete carcinogenesis studies. For the tumor-initiation experiments, the DPM extracts were topically applied in single doses of 100, 500, 1,000, or 2,000 µg/mouse. The high dose (10,000 µg/mouse) was applied in five daily doses of 2,000 µg. One week later, 2 µg of the tumor promoter TPA was applied topically twice weekly. The tumor-promotion experiments used mice treated with 50.5 µg of B[a]P followed by weekly (twice weekly for high dose) topical applications (at the aforementioned doses) of the extracts. For the complete carcinogenesis experiments, the test extracts were applied weekly (twice weekly for the high doses) for 50 to 52 weeks. Only extracts from the Nissan, Oldsmobile, and Caterpillar engines were used in the complete carcinogenesis experiments.

In the tumor-initiation studies, both B[a]P alone and the Nissan engine DPM extract followed by TPA treatment produced a significant increase in tumor (dermal papillomas) incidence at 7 to 8 weeks postapplication. By 15 weeks, the tumor incidence was greater than 90% for both groups. No significant carcinoma formation was noted for mice in the tumor-initiation experiments following exposure to DPM extracts of the other diesel engines, although the Oldsmobile engine DPM extract at 2.0 mg/mouse did produce a 40% papilloma incidence in male mice at 6 mo. This effect, however, was not dose dependent.

B[a]P (50.5 µg/week), coke oven extract (at 1.0, 2.0, or 4.0 mg/week), and the highest dose of roofing tar extract (4.0 mg/week) all tested positive for complete carcinogenesis activity. DPM extracts from only the Nissan, Oldsmobile, and Caterpillar engines were tested for complete carcinogenic potential, and all three proved to be negative using the Sencar mouse assay.

The results of the dermal application experiments by Nesnow et al. (1982) are presented in Table 7-8. The tumor initiation-promotion assay was considered positive if a dose-dependent response was obtained and if at least two doses provided a papilloma-per-mouse value that was three times or greater than that of the background value. Based on these criteria, only emissions

Table 7-8. Dermal tumorigenic and carcinogenic effects of various emission extracts

	Tumor initiation		Complete carcinogenesis	Tumor promotion
Sample	**Papillomas[a]**	**Carcinomas[b]**	**Carcinomas[b]**	**Papillomas[a]**
Benzo[*a*]pyrene	+/+[c]	+/+	+/+	+/+
Topside coke oven	+/+	-/+	ND[d]	ND
Coke oven main	+/+	+/+	+/+	+/+
Roofing tar	+/+	+/+	+/+	+/+
Nissan	+/+	+/+	-/-	ND
Oldsmobile	+/+	-/-	-/-	ND
VW Rabbit	+/+	-/-	I[e]	ND
Mercedes	+/-	-/-	ND	ND
Caterpillar	-/-	-/-	-/-	ND
Residential furnace	-/-	-/-	ND	ND
Mustang	+/+	-/+	ND	ND

[a]Scored at 6 mo.
[b]Cumulative score at 1 year.
[c]Male/female.
[d]ND = Not determined.
[e]I = Incomplete.

Source: Nesnow et al. (1982).

from the Nissan were considered positive. Tumor initiation and complete carcinogenesis assays required that at least one dose produce a tumor incidence of at least 20%. None of the DPM samples yielded positive results based on this criterion.

Kunitake et al. (1986, 1988) evaluated the effects of a dichloromethane extract of DPM obtained from a 1983 MMC M-6D22P 11-L V-6 engine. An acetone solution was applied in 10 doses every other day, followed by promotion with 2.5 µg of TPA three times weekly for 25 weeks. Exposure groups received a total dose of 0.5, 5, 15, or 45 mg of extract. Papillomas were reported in 2 of 50 animals examined in the 45 mg exposure group and in 1 of 48 in the 15 mg group compared with 0 of 50 among controls. Differences, however, were not statistically significant.

7.3.7. Summary and Conclusions of Laboratory Animal Carcinogenicity Studies

As early as 1955, Kotin et al. (1955) provided evidence for tumorigenicity and carcinogenicity of acetone extracts of DPM following dermal application and also provided data suggesting a difference in this potential depending on engine operating mode. Until the early 1980s, no chronic studies assessing inhalation of diesel exhaust, the relevant mode for human exposure, had been reported. Since then long-term inhalation bioassays with diesel exhaust have been carried out in the United States, Germany, Switzerland, and Japan, testing responses of rats, mice, and Syrian hamsters, and to a limited extent cats and monkeys.

It can be reasonably concluded that with adequate exposure, inhalation of diesel exhaust is capable of inducing lung cancer in rats. Responses best fit cumulative exposure (concentration × daily exposure duration × days of exposure). Examination of rat data shown in Table 7-9 indicates a trend of increasing tumor incidence at exposures exceeding 1×10^4 mg·hr/m^3. Exposures greater than approximately this value result in lung particle overload, characterized by slowed particle clearance and lung pathology, as discussed in Chapters 3 and 5, respectively. Tumor induction at high doses may therefore be primarily the result of lung particle overload with associated inflammatory responses. Although tumorigenic responses could not be detected under non-particle-overload conditions, the animal experiments lack sensitivity to determine if a threshold exists. If low-dose effects do occur, it can be hypothesized that the organic constituents are playing a role. See Chapter 7 for a discussion of this issue.

While rats develop adenomas, adenocarcinomas, and adenosquamous cell carcinomas, they also develop squamous keratinizing lesions. This latter spectrum appears for the most part to be peculiar to the rat. In a recent workshop aimed at classifying these tumors (Boorman et al., 1996), it was concluded that when these lesions occur in rats as part of a carcinogenicity study,

Table 7-9. Cumulative (concentration × time) exposure data for rats exposed to whole diesel exhaust

Study	Exposure rate/duration (hr/week, mo)	Total exposure time (hr)	Particle concentration (mg/m^3)	Cumulative exposure ($mg \cdot hr/m^3$)		Tumor incidence (%)[a]
				Per week	Total	
Mauderly et al.	35, 30	4,200	0	0	0	0.9
(1987)	35, 30	4,200	0.35	12.25	1,470	1.3
	35, 30	4,200	3.5	122.5	14,700	3.6
	35, 30	4,200	7.1	248.5	29,820	12.8
Nikula et al. (1995)	80, 23	7,360	0	0	0	1.0
	80, 23	7,360	2.5	200.0	18,400	7.0
	80, 23	7,360	6.5	520.0	47,840	18.0
Heinrich et al.	95, 35	13,300	0	0	0	0
(1986a)	95, 35	13,300	4.24	402.8	56,392	17.8
Heinrich et al.	90, 24	8,640	0	0	0	0
(1995)	90, 24	8,640	0.8	72.0	7,400	0
	90, 24	8,640	2.5	225.0	21,800	5.5
	90, 24	8,640	7.0	630.0	61,700	22.0
Ishinishi et al.	96, 30	11,520	0	0	0	3.3
(1988a)	96, 30	11,520	0.1	9.6	1,152	2.4
(Light-duty engine)	96, 30	11,520	0.4	38.4	4,608	0.8
	96, 30	11,520	1.1	105.6	12,672	4.1
	96, 30	11,520	2.3	220.8	26,496	2.4
(Heavy-duty engine)	96, 30	11,520	0	0	0	0.8
	96, 30	11,520	0.5	48.0	5,760	0.8
	96, 30	11,520	1.0	96.0	11,520	0
	96, 30	11,520	1.8	172.8	20,736	3.3
	96, 30	11,520	3.7	355.2	42,624	6.5

Table 7-9. Cumulative (concentration × time) exposure data for rats exposed to whole diesel exhaust (continued)

Study	Exposure rate/duration (hr/week, mo)	Total exposure time (hr)	Particle concentration (mg/m^3)	Cumulative exposure ($mg \cdot hr/m^3$)		Tumor incidence (%)[a]
				Per week	Total	
Brightwell et al.	80, 24	7,680	0	0	0	1.2
(1989)	80, 24	7,680	0.7	56.0	5,376	0.7
	80, 24	7,680	2.2	176.0	16,896	9.7
	80, 24	7,680	6.6	528.0	50,688	38.5
Kaplan et al. (1983)	140, 15	8,400	0	0	0	0
	140, 15	8,400	0.25	35.0	2,100	3.3
	140, 15	8,400	0.75	105.0	6,300	10.0
	140, 15	8,400	1.5	210.0	12,600	3.3
Iwai et al. (1986)	56, 24	5,376	0	0	0	0
	56, 24	5,376	4.9	274.4	26,342	36.8
Takemoto et al.	16, 18-24	1,152-1,536	0	0	0	0
(1986)	16, 18-24	1,152-1,536	2-4	32-64	3,456-4,608	0
Karagianes et al.	30, 20	2,400	0	0	0	0
(1981)	30, 20	2,400	8.3	249	19,920	16.6
Iwai et al. (1997)	56, 24	5,376	9.4	526	54,704	42
	48, 24	4,992	3.2	154	15,974	12
	54, 24	5,616	5.1	275	28,642	42

[a]Combined data for males and females.

they must be evaluated on a case-by-case basis and regarded as a part of the total biologic profile of the test article. If the only evidence of tumorigenicity is the presence of cystic keratinizing epitheliomas, it may not have relevance to human safety evaluation of a substance or particle. Their use in quantifying cancer potency is even more questionable.

The evidence for response of common strains of laboratory mice exposed under standard inhalation protocols is equivocal. Inhalation of diesel exhaust induced significant increases in lung tumors in female NMRI mice (Heinrich et al., 1986b; Stöber, 1986) and in female Sencar mice (Pepelko and Peirano, 1983). An apparent increase was also seen in female C57BL mice (Takemoto et al., 1986). However, in a repeat of their earlier study, Heinrich et al. (1995) failed to detect lung tumor induction in either NMRI or C57BL/6N mice. No increases in lung tumor rates were reported in a series of inhalation studies using strain A mice (Orthoefer et al., 1981; Kaplan et al., 1982; Kaplan et al., 1983; White et al., 1983). Finally, Mauderly et al. (1996) reported no tumorigenic responses in CD-1 mice exposed under conditions resulting in positive responses in rats. The successful induction of lung tumors in mice by Ichinose et al. (1997a,b) via intratracheal instillation may have been the result of focal deposition of larger doses. Positive effects in Sencar mice may be due to use of a strain sensitive to tumor induction in epidermal tissue by organic agents, as well as exposure from conception, although proof for such a hypothesis is lacking.

Attempts to induce significant increases in lung tumors in Syrian hamsters by inhalation of whole diesel exhaust were unsuccessful (Heinrich et al., 1982, 1986b, 1989b; Brightwell et al., 1986). However, hamsters are considered to be relatively insensitive to lung tumor induction. For example, while cigarette smoke, a known human carcinogen, was shown to induce laryngeal cancer in hamsters, the lungs were relatively unaffected (Dontenwill et al., 1973).

Neither cats (Pepelko and Peirano, 1983 [see Chapter 7]) nor monkeys (Lewis et al., 1986) developed tumors following 2-year exposure to diesel exhaust. The duration of these exposures, however, was likely to be inadequate for these two longer-lived species, and group sizes were quite small. Exposure levels were also below the maximum tolerated dose (MTD) in the monkey studies and, in fact, only borderline for detection of lung tumor increases in rats.

Long-term exposure to diesel exhaust filtered to remove particulate matter failed to induce lung tumors in rats (Heinrich et al., 1986b; Iwai et al., 1986; Brightwell et al., 1989), or in Syrian hamsters (Heinrich et al., 1986b; Brightwell, 1989). A significant increase in lung carcinomas was reported by Heinrich et al. (1986b) in NMRI mice exposed to filtered exhaust. However, in a more recent study the authors were unable to confirm earlier results in either NMRI or C57BL/6N mice (Heinrich et al., 1995). Although filtered exhaust appeared to potentiate the carcinogenic effects of DEN (Heinrich et al., 1982), nevertheless, because of the lack of positive data in rats

and equivocal or negative data in mice, it can be concluded that filtered exhaust is either not carcinogenic or has a low cancer potency.

Kawabata et al. (1986) demonstrated the induction of lung tumors in Fischer 344 rats following intratracheal instillation of DPM. Rittinghausen et al. (1997) reported an increase in cystic keratinizing epitheliomas following intratracheal instillation of rats with either original DPM or DPM extracted to remove the organic fraction, with the unextracted particles inducing a slightly greater effect. Grimmer et al. (1987) showed not only that an extract of DPM was carcinogenic when instilled in the lungs of rats, but also that most of the carcinogenicity resided in the portion containing PAHs with four to seven rings. Intratracheal instillation did not induce lung tumors in Syrian hamsters (Kunitake et al., 1986; Ishinishi et al., 1988b).

Dermal exposure and s.c. injection in mice provided additional evidence for tumorigenic effects of DPM. Particle extracts applied dermally to mice have been shown to induce significant skin tumor increases in two studies (Kotin et al., 1955; Nesnow et al., 1982). Kunitake et al. (1986) also reported a marginally significant increase in skin papillomas in ICR mice treated with an organic extract from an HD diesel engine. Negative results were reported by Depass et al. (1982) for skin-painting studies using mice and acetone extracts of DPM suspensions. However, in this study the exhaust particles were collected at temperatures of 100 °C, which would minimize the condensation of vapor-phase organics and, therefore, reduce the availability of potentially carcinogenic compounds that might normally be present on diesel exhaust particles. A significant increase in the incidence of sarcomas in female C57Bl mice was reported by Kunitake et al. (1986) following s.c. administration of LD DPM extract at doses of 500 mg/kg. Takemoto et al. (1988) provided additional data for this study and reported an increased tumor incidence in the mice following injection of LD engine DPM extract at doses of 100 and 500 mg/kg. Results of i.p. injection of DPM or DPM extracts in strain A mice were generally negative (Orthoefer et al., 1981; Pepelko and Peirano, 1983), suggesting that the strain A mouse may not be a good model for testing diesel emissions.

Results of experiments using tumor initiators such as DEN, B[a]P, DPN, or DBA (Brightwell et al., 1986; Heinrich et al., 1986b; Takemoto et al., 1986) were generally inconclusive regarding the tumor-promoting potential of either filtered or whole diesel exhaust. A report by Heinrich et al. (1982), however, indicated that filtered exhaust may promote the tumor-initiating effects of DEN in hamsters.

Several reports (Wong et al., 1986; Bond et al., 1990) affirm observations of the potential carcinogenicity of diesel exhaust by providing evidence for DNA damage in rats. These findings are discussed in more detail in Section 3.6. Evidence for the mutagenicity of organic agents present in diesel engine emissions is also provided in Chapter 4.

Evidence for the importance of the carbon core was initially provided by studies of Kawabata et al. (1986), which showed induction of lung tumors following intratracheal instillation of carbon black that contained no more than traces of organics, and studies of Heinrich (1990) that indicated that exposure via inhalation to carbon black (Printex 90) particles induced lung tumors at concentrations similar to those effective in DPM studies. Additional studies by Heinrich et al. (1995) and Nikula et al. (1995) confirmed the capability of carbon particles to induce lung tumors. Induction of lung tumors by other particles of low solubility such as titanium dioxide (Lee et al., 1986) confirmed the capability of particles to induce lung tumors. Pyrolyzed pitch, on the other hand, essentially lacking a carbon core but having much higher PAH concentrations than DPM, also was effective in tumor induction (Heinrich et al., 1986a, 1994).

The relative importance of the adsorbed organics, however, remains to be elucidated and is of some concern because of the known carcinogenic capacity of some of these chemicals. These include polycyclic aromatics as well as nitroaromatics, as described in Chapter 2. Organic extracts of particles also have been shown to induce tumors in a variety of injection, intratracheal instillation, and skin-painting studies, and Grimmer et al. (1987) have, in fact, shown that the great majority of the carcinogenic potential following instillation resided in the fraction containing four- to seven-ring PAHs.

In summary, based on positive inhalation and intratracheal instillation data in rats and on i.p. injection or skin painting in mice, and supported by positive mutagenicity studies, the evidence for carcinogenicity of diesel exhaust is considered to be adequate. The contribution of the various fractions of diesel exhaust to the carcinogenic response is less certain. Exposure to filtered exhaust generally failed to induce lung tumors. The presence of known carcinogens adsorbed to diesel particles and the demonstrated tumorigenicity of particle extracts in a variety of injection, instillation, and skin-painting studies indicate a carcinogenic potential for the organic fraction. Studies showing that insoluble particles (e.g., carbon black, TiO_2) can also induce tumors, on the other hand, have provided definitive evidence that the carbon core of the diesel particle is primarily instrumental in the carcinogenic response observed in rats under sufficient exposure conditions. The ability of diesel exhaust to induce lung tumors at non-particle-overload conditions, and the relative contribution of the particles' core versus the particle-associated organics (if effects do occur at low doses) remains to be determined.

7.4. MODE OF ACTION OF DIESEL EMISSION-INDUCED CARCINOGENESIS

As noted in Chapter 2, diesel exhaust (DE) is a complex mixture that includes a vapor phase and a particle phase. The particle phase consists of an insoluble carbon core with a large number of organic compounds, as well as inorganic compounds such as sulfates, adsorbed to the

particle surface. Some of the semivolatile and particle-associated compounds, in particular PAHs, nitro-PAHs, oxy-PAHs, and oxy-nitro-PAHs (Scheepers and Bos, 1992), are considered likely to be carcinogenic in humans. The vapor phase also contains a large number of organic compounds, including several known or probable carcinogens such as benzene and 1,3-butadiene. Since exposure to the vapor phase alone, even at high concentrations, failed to induce lung cancer in laboratory animals (Heinrich et al., 1986), discussion will focus on the particulate matter phase. Additive or synergistic effects of vapor-phase components, however, cannot be totally discounted, since chronic inhalation bioassays involving exposure to diesel particles alone have not been carried out.

Several hypotheses regarding the primary mode of action of diesel exhaust have been proposed. Initially it was generally believed that cancer was induced by particle-associated organics acting via a genotoxic mechanism. By the late 1980s, however, studies indicated that carbon particles virtually devoid of organics could also induce lung cancer at sufficient inhaled concentrations (Heinrich, 1990). This finding provided support for a hypothesis originally proposed by Vostal (1986) that induction of lung tumors arising in rats exposed to high concentrations of diesel exhaust is related to overloading of normal lung clearance mechanisms, accumulation of particles, and cell damage followed by regenerative cell proliferation. The action of particles is therefore mediated by epigenetic mechanisms that can be characterized more by promotional than initiation stages of the carcinogenic process. More recently several studies have focused upon the production of reactive oxygen species generated from particle-associated organics, which may induce oxidative DNA damage at exposure concentrations lower than those required to produce lung particle overload. Since it is likely that more than one of these factors is involved in the carcinogenic process, a key consideration is their likely relative contribution at different exposure levels. The following discussion will therefore consider the possible relationship of the organic components of exhaust, inflammatory responses associated with lung particle overload, reactive oxygen species, and physical characteristics of diesel particles to cancer induction, followed by a hypothesized mode of action, taking into account the likely contribution of the factors discussed.

7.4.1. Potential Role of Organic Exhaust Components in Lung Cancer Induction

More than 100 carcinogenic or potentially carcinogenic components have been specifically identified in diesel emissions, including various PAHs and nitroarenes such as 1-nitropyrene (1-NP) and dinitropyrenes (DNPs). The majority of these compounds are adsorbed to the carbon core of the particulate phase of the exhaust and, if desorbed, may become available for biological processes such as metabolic activation to mutagens. Among such compounds

identified from diesel exhaust are benzo(*a*)pyrene (B[*a*]P), dibenz[*a,h*]anthracene, pyrene, chrysene, and nitroarenes such as 1-NP, 1,3-DNP, 1,6-DNP, and 1,8-DNP, all of which are mutagenic, carcinogenic, or implicated as procarcinogens or cocarcinogens (Stenback et al., 1976; Weinstein and Troll, 1977; Thyssen et al., 1981; Pott and Stöber, 1983; Howard et al., 1983; Hirose et al., 1984; Nesnow et al., 1984; El-Bayoumy et al., 1988). More recently Enya et al. (1997) reported isolation of 3-nitrobenzanthrone, one of the most powerful direct-acting mutagens known to date, from the organic extracts of diesel exhaust.

Grimmer et al. (1987) separated diesel exhaust particle extract into a water- and a lipid-soluble fraction, and the latter was further separated into a PAH-free, a PAH-containing, and a polar fraction by column chromatography. These fractions were then tested in Osborne-Mendel rats by pulmonary implantation at doses corresponding to the composition of the original diesel exhaust. The water-soluble fraction did not induce tumors; the incidences induced by the lipid-soluble fractions were 0% with the PAH-free fraction, 25% with the PAH and nitro-PAH-containing fractions, and 0% with the polar fraction. The PAH and nitro-PAH-containing fraction, comprising only 1% by weight of the total extract, was thus shown to be responsible for most, if not all, of the carcinogenic activity.

Exposure of rats by inhalation to 2.6 mg/m^3 of an aerosol of tar-pitch condensate with no carbon core but containing 50 µg/m^3 benzo[*a*]pyrene along with other PAHs for 10 months induced lung tumors in 39% of the animals. The same amount of tar-pitch vapor condensed onto the surface of carbon black particles at 2 and 6 mg/m^3 resulted in tumor rates that were roughly two times higher (89% and 72%). Since exposure to 6 mg/m^3 carbon black almost devoid of extractable organic material induced a lung tumor rate of 18%, the combination of PAHs and particles increases their effectiveness (Heinrich et al., 1994). While this study shows the tumor-inducing capability of PAHs resulting from combustion, it should be noted that the benzo[*a*]pyrene content in the coal-tar pitch was about three orders of magnitude greater than in diesel soot. Moreover, because organics are present on diesel particles in a thinner layer and the particles are quite convoluted, they may be more tightly bound and less bioavailable. Nevertheless, these studies provide evidence supporting the involvement of organic constituents of diesel particles in the carcinogenic process.

Exposure of humans to related combustion emissions provides some evidence for the involvement of organic components. Mumford et al. (1989) reported greatly increased human lung cancer mortality in Chinese communes burning so-called smoky coal, but not wood, in unvented open-pit fires used for heating and cooking. Although particle concentrations were similar, PAH levels were five to six times greater in the air of communes burning smoky coal.

Coke oven emissions, containing high concentrations of PAHs but lacking an insoluble carbon core, have also been shown to be carcinogenic in humans (Lloyd, 1971).

Adsorption of PAHs to a carrier particle such as hematite, CB, aluminum, or titanium dioxide enhances their carcinogenic potency (Farrell and Davis, 1974). As already noted, adsorption to carbon particles greatly enhanced the tumorigenicity of pyrolyzed pitch condensate containing B[*a*]P and other aromatic carcinogens (Heinrich et al., 1995). The increased effectiveness can be partly explained by more efficient transport to the deep lung. Slow release also enhances residence time in the lungs and prevents overwhelming of activating pathways. As discussed in Chapter 3, free organics are likely to be rapidly absorbed into the bloodstream, which may explain why the vapor-phase component of exhaust is relatively ineffective in the induction of pathologic or carcinogenic effects.

Even though the organic constituents may be tightly bound to the particle surface, significant elution is still likely because particle clearance half-times are nearly 1 year in humans (Bohning et al., 1982). Furthermore, Gerde et al. (1991) presented a model demonstrating that large aggregates of inert dust containing crystalline PAHs are unlikely to form at doses typical of human exposure. This allows the particles to deposit and react with the surrounding lung medium, without interference from other particles. Particle-associated PAHs can then be expected to be released more rapidly from the particles. Bond et al. (1984) provided evidence that alveolar macrophages from beagle dogs metabolized B[*a*]P coated on diesel particles to proximate carcinogenic forms. Unless present on the particle surface, B[*a*]P is more likely to pass directly into the bloodstream and escape activation by phagocytic cells.

The importance of DE-associated PAHs in the induction of lung cancer in humans may be enhanced because of the possibility that the human lung is more sensitive to these compounds than are rat lungs. Rosenkranz (1996) summarized information indicating that in humans and mice, large proportions of lung cancers contain both mutated *p*53 suppressor genes and K-*ras* genes. Induction of mutations in these genes by genotoxins, however, is much lower in rats than in humans or mice.

B[*a*]P, although only one of many PAHs present in diesel exhaust, is the one most extensively studied. Bond et al. (1983, 1984) demonstrated metabolism of particle-associated B[*a*]P and free B[*a*]P by alveolar macrophages (AM) and by type II alveolar cells. The respiratory tract cytochrome P-450 systems have an even greater concentration in the nonciliated bronchiolar cells (Boyd, 1984). It is worthy to note that bronchiolar adenomas that develop following diesel exposure have been found to resemble both Type II and nonciliated bronchiolar cells. It should also be noted that any metabolism of procarcinogens by these latter two cell types probably involves the preextraction of carcinogens in the extracellular lining fluid and/or other

endocytotic cells, since they are not especially important in phagocytosis of particles. Thus, bioavailability is an important issue in assessing the relative importance of PAHs.

Additionally, a report by Borm et al. (1997) indicates that incubating rat lung epithelial-derived cells with human PMNs (either unactivated or activated by preexposure to phorbol myristate acetate) increases DNA adduct formation caused by exposure to benzo[*a*] pyrene; addition of more activated PMN in relation to the number of lung cells further increased adduct formation in a dose-dependent manner. The authors suggest that "an inflammatory response in the lung may increase the biologically effective dose of polycyclic aromatic hydrocarbons (PAHs), and may be relevant to data interpretation and risk assessment of PAH-containing particles." These data raise the possibility that DE exposure at low concentrations may result in levels of neutrophil influx that would not necessarily be detectable via histopathological examination as acute inflammation, but which might be effective at amplifying any potential diesel exhaust genotoxic effect.

Nitro-PAHs have also been implicated as potentially involved in diesel-exhaust-induced lung cancer. Although the nitro-PAH fraction of diesel was less effective than PAHs in the induction of lung cancer when implanted into the lungs of rats (Grimmer et al., 1987), in a study of various extracts of diesel exhaust particles, 30%-40% of the total mutagenicity could be attributed to a group of six nitroarenes (Salmeen et al., 1984). Moreover, Gallagher et al. (1994) reported results suggesting that DNA adducts are formed from nitro-PAHs present in DNA and may play a role in the carcinogenic process. Nitroarenes, however, quantitatively represent a very small percentage of diesel particle extract (Grimmer et al., 1987), making their role in the tumorigenic response uncertain.

The induction of DNA adducts in humans occupationally exposed to diesel exhaust indicates the likelihood that PAHs are participating in the tumorigenic response, and that these effects can occur at exposure levels less than those required to induce lung particle overload. Distinct adduct patterns were found among garage workers occupationally exposed to diesel exhaust when compared to nonexposed controls (Nielsen and Autrup, 1994). Furthermore, the findings were concordant with the adduct patterns observed in groups exposed to low concentrations of PAHs from combustion processes. Hemminki et al. (1994) also reported significantly elevated levels of DNA adducts in lymphocytes from garage workers with known diesel exhaust exposure compared to unexposed mechanics. Hou et al. (1995) found elevated adduct levels in bus maintenance workers exposed to diesel exhaust. Although no difference in mutant frequency was observed between the groups, the adduct levels were significantly different (3.2 vs. 2.3×10^{-8}). Nielsen et al. (1996) measured three biomarkers in DE-exposed bus garage workers: lymphocyte DNA adducts, hydroxyethylvaline adducts in hemoglobin, and

1-hydroxypyrene in urine. Significantly increased levels were reported for all three. Qu et al. (1996) detected increased adduct levels, as well as increases in some individual adducts, in the blood of underground coal miners exposed to DE.

7.4.2. Role of Inflammatory Cytokines and Proteolytic Enzymes in the Induction of Lung Cancer by Diesel Exhaust

It is well recognized that the deposition of particles in the lung can result in the efflux of polymorphonuclear leucocytes (PMNs) from the vascular compartment into the alveolar space compartment in addition to expanding the AM population size. Following acute exposures, the influx of the PMNs is transient, lasting only a few days (Adamson and Bowden, 1978; Bowden and Adamson, 1978; Lehnert et al., 1988). During chronic exposure the numbers of PMNs lavaged from the lungs of diesel-exposed rats generally increased with increasing exposure duration and inhaled diesel particulate matter (DPM) concentration (Strom, 1984). Strom (1984) also found that PMNs in diesel-exposed lungs remained persistently elevated for at least 4 months after cessation of exposure, a potential mechanism that may be related to an ongoing release of phagocytized particles. Evidence in support of this possibility was reported by Lehnert et al. (1989) in a study in which rats were intratracheally instilled with 0.85, 1.06, or 3.6 mg of polystyrene particles. The PMNs were not found to be abnormally abundant during the clearance of the two lower lung burdens, but they became progressively elevated in the lungs of the animals in which alveolar-phase clearance was inhibited. Moreover, the particle burdens in the PMNs became progressively greater over time. Such findings are consistent with an ongoing particle relapse process, in which particles released by dying phagocytes are ingested by new ones.

The inflammatory response, characterized by efflux of PMNs from the vascular compartment, is mediated by inflammatory chemokines. Driscoll et al. (1996) reported that inhalation of high concentrations of carbon black stimulated the release of macrophage inflammatory protein 2 (MIP-2) and monocyte chemotactic protein 1 (MCP-1). They also reported a concomitant increase in hprt mutants. In a following study it was shown that particle exposure stimulates production of tumor necrosis factor TNF-α, an agent capable of activating expression of several proteins that promote both adhesion of leucocytes and chemotaxis (Driscoll et al., 1997). In addition, alveolar macrophages also have the ability to release several other effector molecules or cytokines that can regulate numerous functions of other lung cells, including their rates of proliferation (Bitterman et al., 1983; Jordana et al., 1988; Driscoll et al., 1996).

Another characteristic of AMs and PMNs under particle overload conditions is the release of a variety of potentially destructive hydrolytic enzymes, a process known to occur simultaneously with the phagocytosis of particles (Sandusky et al., 1977). The essentially

continual release of such enzymes during chronic particle deposition and phagocytosis in the lung may be detrimental to the alveolar epithelium, especially to Type I cells. Evans et al. (1986) showed that injury to Type I cells is followed shortly thereafter by a proliferation of Type II cells. Type II cell hyperplasia is a common feature observed in animals that have received high lung burdens of various types of particles, including unreactive polystyrene microspheres. Exaggerated proliferation as a repair or defensive response to DPM deposition may have the effect of amplifying the likelihood of neoplastic transformation in the presence of carcinogens beyond that which would normally occur with lower rates of proliferation, assuming an increase in the cycling of target cells and the probability of a neoplastic-associated genomic disturbance.

7.4.3. Role of Reactive Oxygen Species in Lung Cancer Induction by Diesel Exhaust

Phagocytes from a variety of species produce elevated levels of oxidant reactants in response to challenges, with the physiochemical characteristics of a phagocytized particle being a major factor in determining the magnitude of the oxidant-producing response. Active oxygen species released by the macrophages and lymphatic cells can cause lipid peroxidation in the membrane of lung epithelial cells. These lipid peroxidation products can initiate a cascade of oxygen free radicals that progress through the cell to the nucleus, where they damage DNA. If this damage occurs during the epithelial cell's period of DNA synthesis, there is some probability that the DNA will be replicated unrepaired (Lechner and Mauderly, 1994). The generation of reactive oxygen species by both AMs and PMNs should therefore be considered as one potential factor of what probably is a multistep process that culminates in the development of lung tumors in response to chronic deposition of DPM.

Even though products of phagocytic oxidative metabolism, including superoxide anions, hydrogen peroxide, and hydroxyl radicals, can kill tumor cells (Klebanoff and Clark, 1978), and the reactive oxygen species can peroxidize lipids to produce cytotoxic metabolites such as malonyldialdehyde, some products of oxidative metabolism apparently can also interact with DNA to produce mutations. Cellular DNA is damaged by oxygen free radicals generated from a variety of sources (Ames, 1983; Trotter, 1980). Along this line, Weitzman and Stossel (1981) found that human peripheral leukocytes are mutagenic in the Ames assay. This mutagenic activity was related to PMNs and blood monocytes; blood lymphocytes alone were not mutagenic. These investigators speculated that the mutagenic activity of the phagocytes was a result of their ability to produce reactive oxygen metabolites, inasmuch as blood leukocytes from a patient with chronic granulomatous diseases, in which neutrophils have a defect in the NADPH oxidase generating system (Klebanoff and Clark, 1978), were less effective in producing mutations than were normal leukocytes. Of related significance, Phillips et al. (1984) demonstrated that the incubation of

Chinese hamster ovary cells with xanthine plus xanthine oxidase (a system for enzymatically generating active oxygen species) resulted in genetic damage hallmarked by extensive chromosomal breakage and sister chromatid exchange and produced an increase in the frequency of thioguanidine-resistant cells (HGPRT test). Aside from interactions of oxygen species with DNA, increasing evidence also points to an important role of phagocyte-derived oxidants and/or oxidant products in the metabolic activation of procarcinogens to their ultimate carcinogenic form (Kensler et al., 1987).

Hatch and co-workers (1980) have demonstrated that interactions of guinea pig AMs with a wide variety of particles, such as silica, metal oxide-coated fly ash, polymethylmethacrylate beads, chrysotile asbestos, fugitive dusts, polybead carboxylate microspheres, glass and latex beads, uncoated fly ash, and fiberglass increase the production of reactive oxygen species. Similar findings have been reported by numerous investigators for human, rabbit, mouse, and guinea pig AMs (Drath and Karnovsky, 1975; Allen and Loose, 1976; Beall et al., 1977; Lowrie and Aber, 1977; Miles et al., 1977; Rister and Baehner, 1977; Hoidal et al., 1978). PMNs are also known to increase production of superoxide radicals, hydrogen peroxide, and hydroxyl radicals in response to membrane-reactive agents and particles (Goldstein et al., 1975; Weiss et al., 1978; Root and Metcalf, 1977). Although these responses may occur at any concentration, they are likely to be greatly enhanced at high exposure concentrations with slowed clearance and lung particle overload.

Reactive oxygen species can also be generated from particle-associated organics. Sagai et al. (1993) reported that DPM can nonenzymatically generate active oxygen species (such as superoxide [O_2^-] and hydroxyl radical [$\cdot OH$] in vitro without any biologically activating systems) such as microsomes, macrophages, hydrogen peroxide, or cysteine. Because DPM washed with methanol could no longer produce these radicals, it was concluded that the active components were compounds extractable with organic solvents. However, the nonenzymatic contribution to the DPM-promoted active oxygen production was negligible compared to that generated via an enzymatic route (Ichinose et al., 1997a). They reported that O_2^- and $\cdot OH$ can be enzymatically generated from DPM by the following process. Soot-associated quinone-like compounds are reduced to the semiquinone radical by cytochrome P-450 reductase. These semiquinone radicals then reduce O_2 to O_2^-, and the produced superoxide reduces ferric ions to ferrous ions, which catalyzes the homobiotic cleavage of H_2O_2 dismutated from O_2 by superoxide dismutase or spontaneous reactions to produce $\cdot OH$. According to Kumagai et al. (1997), while quinones are likely to be the favored substrates for this reaction, the participation of nitroaromatics cannot be ruled out.

One of the critical lesions to DNA bases generated by oxygen free radicals is

8-hydroxydeoxyguanosine (8-OHdG). The accumulation of 8-OhdG as a marker of oxidative DNA damage could be an important factor in enhancing the mutation rate leading to lung cancer (Ichinose et al., 1997a). For example, formation of 8-OHdG adducts leads to G:C to T:A transversions unless repaired prior to replication. Nagashima et al. (1995) demonstrated that the production of (8-OHdG) is induced in mouse lungs by intratracheal instillation of DPM. Ichinose et al. (1997b) reported further that while intratracheal instillation of DPM in mice induced a significant increase in lung tumor incidence, comparable increases were not reported when mice were instilled with extracted DPM (to remove organics). Lung injury was also less in the mice instilled with extracted DPM. Moreover, increases in 8-OHdG in the mice instilled with unextracted DPM correlated very well with increases in tumor rates. In a related study, Ichinose et al. (1997a) intratracheally instilled small doses of DPM, 0.05, 0.1, or 0.2 mg weekly for 3 weeks, in mice fed standard or high-fat diets, either with or without β-carotene. High dietary fat enhanced DPM-induced lung tumor incidence, while β-carotene, which may act as a free radical scavenger, partially reduced the tumorigenic response. Formation of 8-OHdG was again significantly correlated with lung tumor incidence in these studies, except at the highest dose. Dasenbrock et al. (1996) reported that extracted DPM, intratracheally instilled into rats (15 mg total dose) induced only marginal increases in lung tumor induction, while unextracted DPM was considerably more effective. While adducts were not measured in this study, it nevertheless provides support for the likelihood that either activation of organic metabolites and/or generation of oxygen free radicals from organics are involved in the carcinogenic process.

Additional support for the involvement of particle-associated radicals in tissue damage was provided by the finding that pretreatment with superoxide dismutase (SOD), an antioxidant, markedly reduced lung injury and death due to instillation of DPM. Similarly Hirafuji et al. (1995) found that the antioxidants catalase, deferoxamine, and MK-447 inhibited the toxic effects of DPM on guinea pig tracheal cells and tissues in vitro.

Although the data presented supported the hypothesis that generation of reactive oxygen species resulting from exposure to DPM is involved in the carcinogenic process, it should be noted that 8-OHdG is efficiently repaired and that definitive proof of a causal relationship in humans is still lacking. It is also uncertain whether superoxide or hydroxyl radicals chemically generated by DPM alone promote 8-OHdG production in vivo and induce lung toxicity, because SOD is extensively located in mammalian tissues. Nevertheless, demonstration that oxygen free radicals can be generated from particle-associated organics, that their presence will induce adduct formation and DNA damage unless repaired, that tumor induction in experimental animals correlated with OhdG adducts, and that treatment with antioxidant limits lung damage, provides

strong support for the involvement of oxygen free radicals in the toxicologic and carcinogenic response to diesel exhaust.

7.4.4. Relationship of Physical Characteristics of Particles to Cancer Induction

The biological potential of inhaled particles is strongly influenced by surface chemistry and character. For example, the presence of trace metal compounds such as aluminum and iron, as well as ionized or protonated sites, is important in this regard (Langer and Nolan, 1994). A major factor is specific surface area (surface area/mg). PMNs characteristically are increased abnormally in the lung by DE exposure, but their presence in the lungs does not appear to be excessive following the pulmonary deposition of even high lung burdens of spherical TiO_2 particles in the 1-2 μm diameter range (Strom, 1984; Lee et al., 1986). In these studies lung tumors were detected only at an inhaled concentration of 250 $\mu g/m^3$. In a more recent study in which rats were exposed to TiO_2 in the 15-40 nm size range, inhibition of particle clearance and tumorigenesis were induced at concentrations of 10 mg/m^3 (Heinrich et al., 1995). Oberdörster and Yu (1990) compared the results of several chronic inhalation studies and found that carcinogenic potency related to specific particle surface area. Heinrich et al. (1995) also found that lung tumor rates increased with specific particle surface area following exposure to diesel exhaust, carbon black, or titanium dioxide, irrespective of particle type. Langer and Nolan (1994) reported that the hemolytic potential of Min-U-Sil15, a silica flour, increased in direct relationship to specific surface area at nominal particle diameters ranging from 0.5 to 20 μm.

Ultrafine particles appear to be more likely to be taken up by lung epithelial cells. Riebe-Imre et al. (1994) reported that CB is taken up by lung epithelial cells in vitro, inducing chromosomal damage and disruption of the cytoskeleton, lesions that closely resemble those present in tumor cells. Johnson et al. (1993) reported that 20-nm polytetrafluoroethylene particles are taken up by pulmonary epithelial cells as well as polymorphonuclear leucocytes, inducing an approximate 4-, 8-, and 40-fold increase in the release of interleukin-1 alpha and beta, inducible nitric oxide synthetase, and macrophage inflammatory protein, respectively.

The carcinogenic potency of diesel particles, therefore, appears to be related, at least to some extent, to their small size and convoluted shape, which results in a large specific particle surface area. While toxicity and carcinogenicity increased with decreasing particle size into the submicron range, it is uncertain if toxic and carcinogenic potential continues to increase as particle size decreases even further. The relationship between particle size and toxicity is of concern because, as noted in Chapter 2, newer engines equipped with more advanced emission controls emit greater numbers of particles in the nanometer size range. Other than disruption of the

cytoskeleton of epithelial cells, there is little information regarding the means by which particle size influences carcinogenicity as well as noncancer toxicity.

7.4.5. Integrative Hypothesis For Diesel-induced Lung Cancer

The induction of lung cancer by large doses of carbon black via inhalation (Heinrich et al., 1995; Mauderly et al., 1991; Nikula et al., 1995) or intratracheal instillation (Kawabata et al., 1994; Pott et al., 1994; Dasenbrock et al., 1996) led to the development of the lung particle overload hypothesis. According to this hypothesis the induction of neoplasia by insoluble, biochemically inert particles is associated with an inhibition of lung particle clearance and the involvement of persistent alveolar epithelial hyperplasia. Driscoll (1995), Driscoll et al. (1996), and Oberdörster and Yu (1990) outlined a proposed mechanism for the carcinogenicity of DE at high doses that emphasizes the role of phagocytic cells. Following exposure, phagocytosis of particles acts as a stimulant for oxidant production and inflammatory cytokine release by lung phagocytes. It was hypothesized that at high particle exposure concentrations the quantity of mediators released by particle-stimulated phagocytes exceeds the inflammatory defenses of the lung (e.g., antioxidants, oxidant-metabolizing enzymes, protease inhibitors, cytokine inhibitors), resulting in tissue injury and inflammation. With continued particle exposure and/or the persistence of excessive particle burdens, there then develops an environment of phagocytic activation, excessive mediator release-tissue injury and, consequently, more tissue injury, inflammation, and tissue release. This is accompanied by cell proliferation. As discussed in a review by Cohen and Ellwein (1991), conceptually, cell proliferation can increase the likelihood that any oxidant-induced or spontaneously occurring genetic damage becomes fixed in a dividing cell and is clonally expanded. The net result of chronic particle exposures sufficient to elicit inflammation and cell proliferation in the rat lung is an increased probability that the genetic changes necessary for neoplastic transformation will occur. A schematic of this hypothesis has been outlined by McClellan (1997) (see Figure 7-5). In support of this hypothesis, it was reported that concentrations of inhaled CB resulted in increased cytokine expression and inflammatory influx of neutrophils (Oberdörster et al., 1995), increased formation of 8-OhdG (Ichinose et al., 1997b), and increase in the yield of hprt mutants, an effect ameliorated by treatment with antioxidants (Driscoll, 1995; Driscoll et al., 1996). Metabolism of carcinogenic

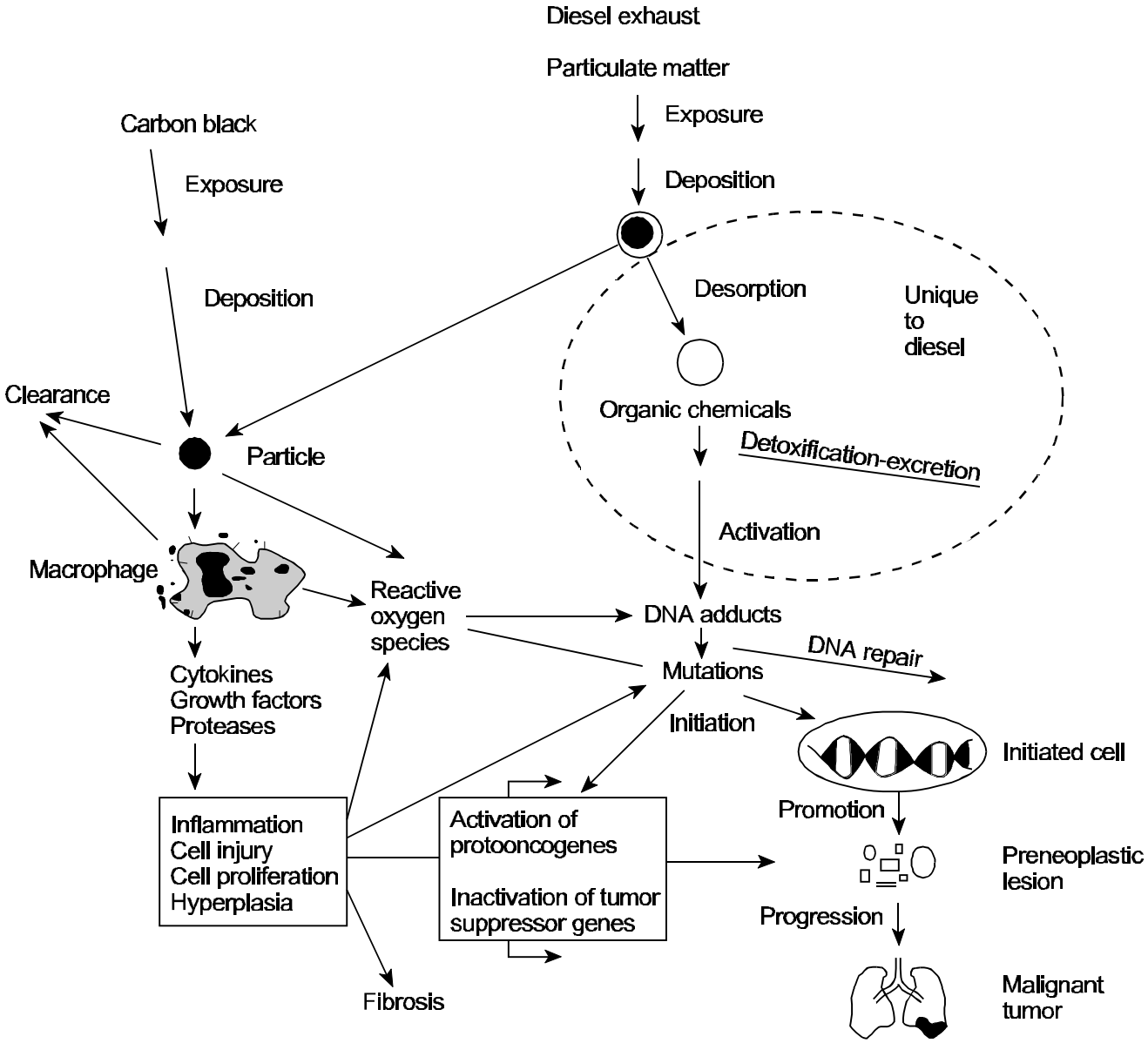


Figure 7-5. Pathogenesis of lung disease in rats with chronic, high-level exposures to particles.

Source: McClellan, 1997.

organics to active forms as well as the generation of reactive oxygen species from certain organic species are likely to contribute to the toxic and carcinogenic process.

At low concentrations, inflammatory effects associated with lung particle overload are generally absent. However, activation of organic carcinogens and generation of oxidants from the organic fraction can still be expected. Actual contribution depends upon elution and the effectiveness of antioxidants. Direct effects of ultrafine diesel particles taken up by epithelial cells are also likely to play a role.

While high-dose induction of cancer is logically explained by this hypothesis, particle overload has not been clearly shown to induce lung cancer in other species. As noted in the quantitative chapter, the relevance of the rat pulmonary response is therefore problematic. The rat pulmonary noncancer responses to DPM, however, have fairly clear interspecies and human parallels. In response to poorly soluble particles such as DPM, human and rats both develop an alveolar macrophage response, accumulate particles in the interstitium, and show mild interstitial fibrosis (ILSI, 1999). Other species (mice, hamsters) also have shown similar noncancer pulmonary responses to DPM, but without accompanying cancer response. The rat response for noncancer pulmonary histopathology, however, seems to be more pronounced compared to humans or other species, i.e., rats appear to be more sensitive. Although many critical elements of interspecies comparison such as the role of airway geometry and patterns of particle deposition need further elucidation, this basic interspecies similarity and greater sensitivity of pulmonary response make pulmonary histopathology in rats a valid basis for noncancer dose-response assessment.

7.4.6. Summary

Recent studies have shown tumor rates resulting from exposures to nearly organic-free CB particles at high concentrations to be similar to those observed for DE exposures, thus providing strong evidence for a particle overload mechanism for DE-induced pulmonary carcinogenesis in rats. Such a mechanism is also supported by the fact that carbon particles per se cause inflammatory responses and increased epithelial cell proliferation and that AM function may be compromised under conditions of particle overload.

The particle overload hypothesis appears sufficient to account for DE-induced lung cancer in rats. However, there is increasing evidence for lung cancer induction in humans at concentrations insufficient to induce lung particle overload (see Chapter 7). Uptake of particles by epithelial cells at ambient or occupational exposure levels, DNA damage resulting from oxygen-free radicals generated from organic molecules, and the gradual in situ extraction and activation of procarcinogens associated with the diesel particles are likely to play a role in this

response. The slower particle clearance rates in humans (up to a year or more) increase the likelihood of significant extraction of organics. This is supported by reports of increased DNA adducts in humans occupationally exposed to diesel exhaust at concentrations unlikely to induce lung particle overload. While these modes of action can be expected to function at lung overload conditions also, they are likely to be overwhelmed by inflammatory associated effects.

The evidence to date indicates that caution must be exercised in extrapolating observations made in animal models to humans when assessing the potential for DE-induced pulmonary carcinogenesis. The carcinogenic response and the formation of DNA adducts in rats exposed to diesel exhaust and other particles at high exposure concentrations may be species-specific and not particle-specific. The likelihood that different modes of action predominate at high and low doses also renders low-dose extrapolation to ambient concentrations uncertain.

7.5. CANCER WEIGHT-OF-EVIDENCE: HAZARD EVALUATION

7.5.1. Cancer Hazard Summary

Diesel engine exhaust is *"highly likely"* to be carcinogenic by the inhalation route of exposure, according to EPA's 1996 Proposed Guidelines for Carcinogen Risk Assessment. The hazard is viewed as being applicable to ambient-environmental exposures. There is no available evidence to evaluate the hazard from other routes of exposure. The "likely" classification generally compares with other agents designated as "B-1 probable human carcinogen" under the EPA's 1986 Guidelines for Carcinogen Risk Assessment, though the overall weight-of-evidence for diesel exhaust (DE) places it at the upper end of this grouping and hence gives it a "highly likely" designation under the proposed Guidelines. The carcinogenic potential of DE is indicated by (1) a consistent statistically increased association between observed lung cancer and DE exposure in certain occupationally exposed workers, (2) the induction of lung cancer in some but not all animal experiments, (3) mutagenic and carcinogenic activity of the particle organic extracts, (4) the presence of individual organic compounds having known mutagenic and/or carcinogenic properties (e.g., PAH derivatives and nitro-PAHs), and (5) limited evidence for the bioavailability of the organics. The mode of action for carcinogenicity in humans is unknown; it is suspected that either the organics, the elemental carbon diesel particle, or both contribute to the carcinogenic activity.

Increases in relative risk for lung cancer have been consistently noted in a number of epidemiologic studies, and causality considerations for this observed association are consistent with DE exposure being causally related to lung cancer. An inability to satisfactorily minimize all confounding, bias, and exposure uncertainties, coupled with the magnitude of the relative risks,

limits the human evidence from being considered sufficient to characterize DE as a "known" human carcinogen.

While lung cancer has been induced experimentally in rats via inhalation of DE at high exposure concentrations, and in rats and mice via intratracheal instillation of diesel particles and particle extracts, these responses appear to be mediated primarily by inflammation and subsequent pathology related to lung particle overload. Because the persistent-chronic overload inflammatory responses in the rat are not seen at lower test exposures (or at ambient DE concentrations), and uncertainty remains whether induction of inflammatory responses in humans will lead to lung cancer, rat bioassay data are not completely irrelevant for human hazard characterization. However, the rat lung cancer response data are unsuitable for estimating human risk at environmental levels of exposure.

The plausibility of an environmental hazard is supported by (1) considering that mutagenic compounds and tumor-initiating carcinogens (e.g., PAH derivatives and nitro-PAHs) are present in small quantities in the DE organic mixture, which qualitatively implies a nonthreshold mode of action for these agents; and (2) noting that there could be little difference between higher-end environmental exposures and some occupational levels where increased relative risks of about 1.4 are seen (e.g., exposure estimates for some truck drivers could be overlapping some environmental estimates, they also may have somewhat higher relative risks). For these reasons, the extrapolation of the occupational hazard to ambient environmental exposures is judged plausible and prudent. In the absence of evidence to the contrary, and recognizing the mutagenic potential of the organics, it would also be feasible to evaluate dose response using linear models, at least at low exposure levels.

Overall, the evidence for a likely human carcinogenic hazard by inhalation is strong, even though inferences are involved. Uncertainties remain, including (1) methodologic limitations in the epidemiologic studies as well as a lack of assured historical exposure data for occupationally exposed cohorts, (2) uncertainties regarding the extent of bioavailability of organic compounds present on diesel particles, and (3) uncertainties regarding the mode of action in humans.

7.5.2. Supporting Information

7.5.2.1. *Human Data*

An increased relative risk for lung cancer and DE exposure has been observed in more than 30 epidemiologic studies. The excess risk is observed in both cohort and case-control study designs. Additionally, consistent and statistically significant elevated pooled relative risks ranging from 1.33 to 1.47 were derived in several meta-analyses. In some studies, the effects of smoking were accounted for and the increased relative risks prevailed. When the meta- analysis focused

only on the smoking-controlled studies, the relative risks tended to increase. A few individual studies had smoking-adjusted relative risks exceeding 1.5 (e.g., Steenland et al., 1990 [RR 1.64, 1.89]). The uncertainties with the epidemiologic data are typical ones including the possibility that chance, bias, or confounding are influencing the observed lung cancer increases. The persistence of this association in so many studies indicates that chance alone is unlikely to account for the observed relation between DE and lung cancer. A causal interpretation for DE is enhanced when the "Hill" causality criteria are evaluated, noting that a weakness or absence in one or several of the criteria does not prevent a causal interpretation. A weakness in the epidemiologic studies is due to the fact that the information from which diesel exposure can be inferred is based on job codes, area descriptions, etc., which are surrogates for the true underlying exposure. This can lead to "nondifferential" misclassification of exposure, and while unlikely, might result in a spurious risk estimate in any one study. It is even more unlikely, however, that it would bias a sufficient number of studies in a uniform direction to account for the persistent aggregate association observed. Moreover, any bias would likely be toward a lower risk estimate. In those studies where the confounding effect of smoking was controlled, there remains the suspicion that the statistical adjustment for smoking may not be completely effective, and residual confounding by smoking may persist to bias the correlation of DE exposure with lung cancer occurrence.

7.5.2.2. *Animal Data*

Numerous studies have shown that inhalation of DE and intratracheal instillation of diesel particles or particle extract result in the induction of lung cancer in rats. Although evidence of lung cancer induction from DE in mice via inhalation exposure is equivocal, positive results have been obtained by intratracheal instillation of diesel particles. Attempts to induce lung cancer in Syrian hamsters by inhalation of DE have been unsuccessful, but this species is known to be resistant to the induction of lung cancer. Although cats and monkeys have been exposed to DE, the durations of exposure were inadequate to evaluate carcinogenicity. As supported by an expert panel (ILSI, 1998), the high-dose rat data are unsuitable for predicting a low-exposure human risk. Because it is unknown whether high lung burdens of poorly soluble particles (e.g., diesel particles) can lead to lung cancer in humans via mechanisms similar to those of the rat, there are insufficient data to conclude that the rat response is completely irrelevant for human hazard identification. Intratracheal instillation studies in rats and mice reveal that diesel particles as well as the particle organic extracts can elicit a lung cancer response.

7.5.2.3. *Other Key Data*

Organic extracts of DE particles have been shown to induce tumors in mice, both by skin painting and subcutaneous injection, and to be mutagenic in several test systems. Additionally, a number of PAHs and nitro-PAHs present on diesel particles as well as in the vapor phase are known to be mutagenic and/or carcinogenic. Further evidence for the presence of carcinogenic agents in DE is provided by the reported induction of dermal tumors following skin painting of diesel particle extracts.

7.5.2.4. *Mode of Action*

The mode of action for DE carcinogenicity, especially at nonparticle overload exposure conditions, remains to be established. There is some evidence and thus plausibility that diesel particles as well as particle-associated organics are involved in the carcinogenic process. The rat model shows that at high-exposure concentrations, particle-overload-induced inflammatory responses, associated DNA damage, and rapid cell turnover are likely the primary factors responsible for lung cancer induction. It is not known whether humans have a similar response pattern at high exposures, though it has not been historically observed. At low exposure levels, cancer induction is more likely to be due to organic compounds, although there is some evidence that ultrafine diesel particles at low concentrations are ingested by epithelial cells and induce DNA damage. DNA damage in blood cells of occupationally exposed workers indicates at least some degree of elution of organic compounds from the particle and subsequent entry into the bloodstream. Studies have also suggested that bioavailability may be greater at low-exposure concentrations because the particles are not aggregated. A significant percentage of particles in humans are deposited at the branchings of small airways rather than alveoli, and the residence time of organic compounds eluted at those locations is greater, increasing the likelihood of metabolism to an activated state. A variety of carcinogenic compounds (e.g., PAH derivatives and nitro-PAHs), a number of which are mutagens and carcinogens, are present on the diesel particle. It has also been shown that reactive oxygen species capable of damaging DNA are generated by the metabolism of DE organics with quinone-like structures.

7.6. DISCUSSION OF THE ROLE OF DIESEL EXHAUST IN THE OVERALL PICTURE OF PM_{10}

It is very difficult to assess exposure to diesel emissions because they are highly complex mixtures and constitute only a small portion of a broader mix of air pollutants. For example, combustion of other materials, such as fossil fuel and tobacco, produces many of the same chemical components present in diesel emissions; furthermore, both natural and manmade sources

of respirable particles are common. No single constituent of diesel exhaust serves as a unique marker of exposure.

Whether air pollution contributes to the occurrence of lung cancer is a matter of wide debate. Ambient air in urban or industrialized areas can be contaminated by chemicals, some of which are definitely known to be carcinogenic. Known carcinogens that occur in ambient air include arsenic, asbestos, benzene, cadmium, and polycyclic aromatic hydrocarbons. However, the information on ambient exposure is sparse. Detailed measurements of such substances over long exposure periods and for large geographic areas are rarely available. Many descriptive epidemiologic studies demonstrate increased lung cancer risk in urban and industrialized areas (Hemminki, 1994). Frequently, those differences have been partially explained by differences in air pollution; however, such correlations might have other explanations and do not represent final conclusive evidence. The coincidence of diesel exhaust exposure and air pollution in urban airsheds poses important questions about whether the observed association of an increased lung cancer risk in urban and industrialized areas can be attributed to diesel exhaust exposure.

The contribution of diesel particles to PM_{10} (particles $\leq$10 *u*m diameter) are difficult to determine, although most estimates indicate they constitute only a small fraction. For example, in an analysis conducted in the Los Angeles basin in the early 1980s, diesel emissions accounted for approximately 3% of the mass of PM_{10}. Because 90% of diesel particles are less than 1 *um* diameter they make up a larger percentage of fine and ultrafine ambient PM. For example, the EPA has estimated that diesel particles accounts for 5.7% of all $PM_{2.5}$ and 21% of $PM_{2.5}$ excluding natural and fugitive dust sources (see Chapter 2 for details). Since smaller particles appear to be more toxic/carcinogenic, the size distribution as well as mass of diesel particles relative to other PM are important considerations in any attempt to estimate the contribution of DE to PM induced toxicity and/or carcinogenicity.

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